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Cycling of Xenobiotics through Marine and Estuarine Sediments

University of West Florida, Pensacola

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CYCLING OF XENOBIOTICS THROUGH MARINE AND ESTUARINE SEDIMENTS

Extracted from cited papers by

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GRANT NO. R804458

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16. ABSTRACT

The results of five broadly defined projects are reported. Cycling of xenobiotics was studied with a photo-bioassay system that used time-lapse photography to evaluate effects of Kepone and sodium pentachlorophenate on feeding activity of the lugworm, Arenicola cristata. Radio-labeled methyl parathion was used to demonstrate fate and effect in microcosms inhabited by lugworms. Uptake and depuration of chrysene by lugworms was evaluated in a flow-through system. A toxic sediment bioassay system was developed to provide a means to test effects of dredge spoil. Kepone-sorbed sediment and dredge spoil from James River and Houston Ship Channel were tested for 28 days. Long-term tests (100 days), with the same systems, were used to evaluate effects of a specific drilling mud from an active exploratory platform. Predator-prey tests of sublethal effects of xenobiotics demonstrated effect in one-prey and two-prey systems. Evaluation of sublethal effects, such as avoidance of pollution gradients, was studied in a trough-type avoidance-response system. The system recorded responses automatically and independently of an observer. Tests with pinfish demonstrated that they will avoid chlorine-produced oxidants. Usefulness of small-scale microcosms was evaluated by developing methods to culture polychaetes and crustaceans. Various aspects of the biology of selected species were studied.

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Although the research described in this report has been funded wholly or in part by the United States Environmental Protection Agency under Grant agreement number R804458 to C.N. D'Asaro, the University of West Florida, it has not been subjected to the Agency's required peer and administrative review and, therefore, does not necessarily reflect the view of the Agency and no official endorsement should be inferred.

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants required that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze (ERL,GB), contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities of organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environment.

The assessment of the potential impact of environmental contaminants depends on the accurate measurement of the fate and effects of these pollutants in both field and laboratory. This report describes research directed toward developing methods to provide more realistic evaluators, other than acute and chronic toxicity tests, for a xenobiotic's fate and effect in estuarine and marine systems. This information will be considered for future inclusion in the testing requirements in support of the U.S. Environmental Protection Agency regulatory programs.

Henry F. Enos
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ABSTRACT

The results of five broadly defined projects are reported.

Cycling of xenobiotics was studied with a photo-bioassay system, that used time-lapse photography to evaluate effects of Kepone and sodium pentachlorophenate on feeding activity of the lugworm, Arenicola cristata. Radio-labeled methyl parathion was used to demonstrate fate and effect in microcosms inhabited by lugworms. Uptake and depuration of chrysene by lugworms was evaluated in a flow-through system.

A toxic sediment bioassay system was developed to provide a means to test effects of dredge spoil. The system included microcosms that held mysid shrimp, Mysidopsis bahia; oysters, Crassostrea virginica; and lugworms, Arenicola cristata. Criteria of effect were survival of mysids, shell deposition and bioaccumulation by oysters, substrate reworking and bioaccumulation by lugworms, and settlement of zooplankton. Kepone-sorbed sediment and dredge spoil from James River and Houston Ship Channel were tested for 28 days. Long-term tests (100 days), with the same systems, were used to evaluate effects of a specific drilling mud from an active exploratory platform.

Predator-prey tests of sublethal effects of xenobiotics demonstrated effect in one-prey and two-prey systems. The effects of methyl parathion on predator-prey relationships between grass shrimp, Palaemonetes pugio; juvenile sheepshead minnows, Cyprinodon variegatus; and gulf killifish, Fundulus grandis, were demonstrated. The effects of methyl parathion on the predator prey relationships between Palaemonetes pugio, and pinfish, Lagodon rhomboides, was also demonstrated. A method that could be used to evaluate effects xenobiotics on predator-prey relationships between cryptically shaded flounder and pinfish prey was developed.

Evaluation of sublethal effects, such as avoidance of pollution gradients, was studied in a trough-type avoidance-response system. The system recorded responses automatically and independently of an observer. Tests with pinfish demonstrated that they will avoid chlorine-produced oxidants. The system was modified to demonstrate toxicant-induced changes in cyclic burrowing activity by pink shrimp, Penaeus duorarum, exposed to methyl parathion.

Usefulness of small-scale microcosms was evaluated by developing methods to culture polychaetes and crustaceans. Various aspects of the biology of selected species were studied.

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INTRODUCTION

Five broadly defined projects were included in the project goals of Grant R804458, "Cycling of Xenobiotics through Marine and Estuarine Sediments." Two primary goals were:

(1) to evaluate cycling of selected xenobiotics or uptake and effect of selected energy-related compounds in experimental systems that included the lugworm, Arenicola cristata; and

(2) to develop a toxic sediment assay system involving the lugworm and other species.

The remaining projects were directed toward developing methods to provide more sensitive evaluators, other than acute and chronic toxicity tests, for a xenobiotic's fate and effect in estuarine and marine ecosystems. Specifically, these were:

(1) development of tests involving estuarine and marine crustaceans and fishes designed to evaluate how exposure to xenobiotics can alter predator-prey relationships,

(2) development and testing of behavioral assays that would provide reliable means to evaluate sublethal effects, such as avoidance; and

(3) establishment of small-scale microcosms that could be used to test fate and effect of various xenobiotics.

Although the five broadly defined projects are divergent, commonalities included either use of systems dominated by lugworms or evaluation of sublethal effects of xenobiotics on marine and estuarine organisms in small-scale microcosms.

CYCLING OF XENOBIOTICS BY THE LUGWORM, ARENICOLA CRISTATA

The impetus to design an assay system involving a lugworm resulted from development of culture methods for that species and recognition that toxicity tests employed by the U.S. Environmental Protection Agency (EPA) for estuarine and marine species do not include an infaunal organism.

BENTHIC PHOTO-BIOASSAY SYSTEM

The first test to include an infaunal representative was Rubinstein's (1979) benthic bioassay, which uses time-lapse photography to measure

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effect of toxicants on feeding behavior of lugworms (Fig. 1). That organism is ideal for this type of test because it is widely distributed in littoral habitats and has major ecological impact due to its ability to recycle sediment and transport xenobiotics into the substrate. The photo-bioassay system constructed was based on the lugworm's habit of creating feeding funnels on the surface of sediment it occupies. Under normal circumstances, active worms, unperturbed by xenobiotics in the water column or sorbed on the sediment, create, enlarge, and recreate obvious funnels. This pattern on the surface of the substrate, which indicates activity of the worm, was monitored by Rubinstein with time-lapse photographs taken at 12-hour intervals for 72 hours. Areas of feeding funnels in exposed and control aquaria were calculated and compared. Initial trials demonstrated that there was no significant difference in reworking activity between replicates under control conditions (Fig. 2). As worms were influenced by xenobiotics, their activity, when compared to controls kept under the same environmental conditions, decreased. For these experiments, the xenobiotic tested was Kepone at measured concentrations of 2.8, 4.5, 6.6, 7.4, and 29.5 µg/ℓ.

Results indicated that A. cristata was sensitive to Kepone at all concentrations tested (Fig. 3). The highest concentration was acutely toxic. Lugworms appeared to be more sensitive to Kepone than many other species normally used in toxicity tests. It appeared that in Kepone-contaminated habitats, the ability of lugworms to rework sediment would be markedly decreased.

CYCLING OF METHYL PARATHION BY LUGWORMS

A second evaluation of cycling of xenobiotics by lugworms (Garnas et al. 1977) was directed toward determining compartmentation and degradation dynamics of methyl parathion in a small-scale microcosm occupied only by the the worm and microorganisms associated with the organic material on which it feeds. Ninety percent of radio-labelled methyl parathion disappeared from the water column in aquaria after 14 days. Movement into the sediment proved to be the major compartmentation phenomenon, with over half of the total radioactivity residing in the sediment after two weeks. The lugworm enhanced movement of radioactivity into the sediment and caused dispersion throughout the sediment. Although volatilization losses were negligible, steadily decreasing mass balance of radioactivity in the system suggested accumulation of unextractable residues in the sediment. Analysis of extractable radioactivity in the sediment and water compartments by thin-layer chromatography and autoradiography demonstrated rapid degradation of methyl parathion into a number of more polar products, including p-nitrophenol and amino-methyl parathion. Although A. cristata was shown to metabolize methyl parathion readily to p-nitrophenol, microbial activity accounted for the majority of biological degradation in the system.

EFFECT OF SODIUM PENTACHLOROPHENATE ON LUGWORM ACTIVITY

The third analysis of effect of xenobiotics on activities of the lugworm was Rubinstein's (1978) evaluation of effect of sodium pentachlorophenate (Na-PCP) on feeding activity. Na-PCP was used because it is an energy-related compound (oil-well drilling fluids) and because it enters estuarine and marine systems occupied by lugworms from numerous non-point sources. Photo-bioassay methods developed by Rubinstein (Fig. 1) were used in this study. Stock solutions of Na-PCP prepared from a commercial bactericide were introduced into experimental aquaria at 45, 80, 156, and 276 µg/ℓ. Comparisons were made between the areas of feeding funnels in exposed and control aquaria. Na-PCP had no marked effect on feeding activity at the lowest concentration tested; however, at the other concentrations, there was significant decrease in activity (Fig 4). Some death occurred at the higher concentration.

UPTAKE AND DEPURATION OF CHRYSENE BY LUGWORMS

The final analysis of cycling of xenobiotics by lugworms evaluated uptake and depuration of chrysene, another energy-related compound (Rubinstein et al. 1980a). Worms were exposed to chrysene at measured concentrations of 0.07, 0.69, and 2.76 µg/ℓ in large wooden tanks in an open system that simulated ambient conditions and the natural habitat (Fig. 5). After 14 days, exposed worms were moved to uncontaminated systems and allowed to depurate for 14 days (considerable death was encountered due to handling in cold weather.) From lowest to highest exposure, lugworms accumulated 65, 516, and 682 µg/ℓ in 14 days (Fig. 6). There was a continued increase in accumulation during that period, so it is probable that had exposure time been increased, higher concentrations of chrysene would have been encountered before equilibrium was reached. Little depuration was observed. This suggested that lugworms are unable to degrade chrysene; thus, there is a good possibility that they have the potential to introduce chrysene into various food chains utilized by man.

TOXIC SEDIMENT BIOASSAY SYSTEM

Many xenobiotics in marine environments have a high affinity for particulate material (especially organics) and thus become sequestered in bottom sediments. Due to increased dredging and maintenance of navigable water, there is a greater need to evaluate impact of toxic sediments on the biota. For that reason, grant-related activities were directed toward developing a flow-through toxicity test that could be used to determine biological effects of contaminated sediments on representative estuarine organisms and to evaluate resiliency of benthic communities exposed to contaminated sediments. The test developed (Rubinstein et al. 1980b) incorporated several established toxicity tests that were modified to examine acute and sublethal effects of dredged sediments on the biota. It was designed to serve as a screening tool to detect potential hazards of dredge spoils prior to disposal in the marine environment.

METHODS DEVELOPMENT WITH KEPONE-SORBED SEDIMENT

The approach was to simulate, then compare certain aspects of the marine environment before and after deposition of spoil material. Small-scale estuarine microcosms were assembled in 10-gallon aquaria that received flowing, unfiltered seawater. Three aquaria received different concentrations of test sediments, while three others remained unperturbed and served as controls. Comparisons were made after 28 days. Organisms included in the test are representative of three environmental compartments affected by dredging activities. Included were mysid shrimp, Mysidopsis bahia; oysters, Crassostrea virginica; and lugworms, Arenicola cristata. Test criteria used to identify effect were: (1) survival of mysids; (2) shell deposition and bioaccumulation of known contaminants by oysters; (3) substrate reworking and bioaccumulation by lugworms; and (4) resiliency of the benthic community in terms of numbers and variety of macrofaunal organisms that settled onto test sediments as planktonic larvae within 28 days.

The exposure system employed is shown in Figure 7. Sediments exposed to Kepone at 0.1, 1.0, and 10.0 µg/ℓ were used during evaluation of the method. This was followed by tests with actual dredge spoil from the James River and Houston Ship Channel.

Effect of Kepone-sorbed sediment on mysid survival was time- and dose-dependent (Fig. 8). Oyster shell growth was significantly inhibited (Fig. 9). Lugworms had an increasing dose-dependent relationship in concentration of Kepone. Whole-body residues were 0.043, 0.46, and 1.1 µg/ℓ. Nineteen macrofaunal species from four major taxa were identified. Numbers of species and animals present are shown in Fig. 10. In terms of test criteria, only polychaetes were affected at the highest exposure.

TESTS WITH DREDGE SPOIL

James River sediment did not affect mysids significantly although there was some effect on oysters (Fig. 11). Lugworm substrate reworking was reduced in experimental aquaria. Oysters and lugworms concentrated Kepone (Fig. 12). Little difference was seen in survival of recruited larvae, perhaps because few larvae entered the system during the winter when it was operational.

Houston Ship Channel sediment did not significantly affect mysid survival or oyster shell deposition; nor did lugworm activity or macrofaunal composition vary significantly between control and experimental units.

TESTS WITH DRILLING MUDS

Since the toxic sediment assay system was proven to be effective, a long-term (100-day) toxicity test was conducted to determine effects of a specific drilling mud (Rubinstein et al. 1980c). Drilling muds were obtained weekly from an active exploratory platform and tested within one week of collection

in the system shown in Figure 7. Three dilutions were tested: 10, 30, and 100 mℓ/ℓ. These concentrations represented those expected to occur at intervals of from several meters to several hundred meters from a point source. Mud was added to test aquaria to simulate periodic discharge. The same species previously employed were included in this test, but mysids were exposed for only 10 days.

Mysids exposed in the system were not affected acutely (Fig. 13). Oyster shell growth was inhibited significantly at concentrations of 30 and 100 mℓ/ℓ (Figs. 14 & 15), but there were no deaths. Lugworms were severely affected by exposure to the mud (Fig. 16). Mortalities observed were 75% at 100 mℓ/ℓ, 64% at 30 mℓ/ℓ, and 33% at 10 mℓ/ℓ. Twenty recruited species were present after 100 days (Fig. 17). There was no significant difference between populations in the aquaria. Ba, Cr, and Pb were found to have accumulated significantly in oyster tissue (Fig. 18).

The results indicate that physical as well as chemical properties must be considered before environmental impact of drilling fluids can adequately be assessed. It was also recognized that composition of drilling muds is highly variable; thus, impact should be considered on a case by case basis.

PREDATOR-PREY TESTS

Sublethal concentrations of xenobiotics, especially pesticides, may be expected to affect various aspects of behavior. This was demonstrated by Farr (1977) (partly funded by this grant), who demonstrated that methyl parathion impairs the ability of grass shrimp, Palamonetes pugio, to escape predation by the Gulf killifish, Fundulus grandis. Although F. grandis ate no more exposed than unexposed shrimp, the predators took less time to capture exposed prey. If pesticides have different effects on species in a multiprey system, predators may be expected to consume a higher than normal proportion of affected species. The result would be more rapid accumulation of a xenobiotic.

TWO PREY SYSTEM

Palaemonetes pugio and juvenile sheepshead minnow, Cyprinodon variegatus, were exposed to methyl parathion for 24 hours before introduction of Fundulus grandis, the predator (Farr 1978a). Two experiments were run for five days: a preliminary experiment at 0.475 µg/ℓ, and a definitive experiment which included a carrier control and methyl parathion concentrations of 0.024, 0.119, and 0.475 µg/ℓ.

In the first experiment, when the prey were exposed to the pesticide, Gulf killifish consumed a greater proportion of grass shrimp relative to sheepshead minnows (Fig. 19). Predation was also relatively greater on P. pugio than on C. variegatus, as compared with controls (Fig. 20). The second experiment tested effect of a range of methyl parathion concentrations and of acetone (carrier) on prey consumption. In both control and acetone-control aquaria, the ratio of shrimp to fish increased rapidly during the

test and did not differ, indicating strong predator preferences for sheepshead minnows and no acetone-related effect (Fig. 21). As the concentration of pesticide was increased in test aquaria, the ratio of grass shrimp to sheepshead minnows decreased with time (Fig. 22). Increasing the concentration resulted in increased consumption of grass shrimp relative to fish prey, an obvious example of how a pesticide can alter relative proportions of prey in a predator's diet.

EXPOSED AND CONTROL PREY IN THE SAME SYSTEM

Test systems were modified from the work of Farr (1978a) and focused on the effect of xenobiotics on exposed and control prey in the same system (Cripe and Ashton 1979). Equal numbers of pinfish, Lagodon rhomboides, and toxicant-exposed and control grass shrimp, Palaemonetes pugio, were placed in two replicate tanks containing removable dividers. Approximately 20 minutes after the dividers were removed, surviving shrimp were counted to determine differential predation between exposed and control prey. Prey were pleopod-clipped for identification. Clipping was demonstrated to have no significant effect on predation.

Significantly fewer shrimp survived predation after exposure to 1.2 ppb methyl parathion for 24 hours. Exposure to 1.3 ppb Trithion for 24 and 72 hours produced no significant difference in predation.

CRYPTIC SHADING AND PREDATION

The behavior between a bothid flounder, Paralichthys albigutta, and its prey the pinfish, Lagodon rhomboides, can be exploited to evaluate how a xenobiotic could alter predatory strategy of flounder or avoidance response of pinfish. Before the relationship could be tested, it was necessary to determine the prey's normal response to flounder that exhibited various degrees of cryptic coloration.

For these experiments, models of flounder prey were used (Ashton 1981). Black and white photographs were laminated between plastic and attached to a plastic outline of a flounder. A circular tank was used as the arena (Fig. 23). Ten prey were released from a central holding chamber and photographs were taken at intervals of 0.5, 1.0, and 1.5 minutes to record response to the flounder model. For each control, dark model, and light model treatment ten trials were completed. Position of the school of prey relative to the predator model was calculated. The group response for ten trials at each time-interval was combined for each treatment group. Random versus non-random distribution was tested by Hotelling's one-sample test.

Control data indicated that pinfish were randomly distributed in the absence of a model (Fig. 24). In the case of the light model that represented a cyptically shaded flounder, there was no significant avoidance (Fig. 24). Pinfish swam directly over the model. In the case of the dark model, the pinfish preferred the opposite side of the tank from the model (Fig. 24).

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Pinfish did not swim around the tank as they did in control and light model experiments.

This system is now ready to be tested to determine how sublethal exposure to xenobiotics can modify the antipredator response of the pinfish.

EVALUATION OF SUBLETHAL EFFECTS IN SPECIAL TEST SYSTEMS

AVOIDANCE OF POLLUTION GRADIENTS

It has often been observed that fish and invertebrates avoid pollution gradients. Most apparatus designed to detect avoidance of pollutants by aquatic organisms requires visual observation of the test organisms in steep pollution gradients. The Aquatic Gradient Avoidance Response System (AGARS) was developed to eliminate these limitations (Cripe, 1979a). This system (Fig. 25) allows animals to choose among one uncontaminated zone and three increasingly toxic zones in a gradient trough that is monitored for extended periods by infrared light sources, sensors, and a microprocessor. Data are accumulated hourly and processed by a paper tape reader/calculator/plotter system that records the time test animals remain in each zone and compares behavior before and during test exposures. Initial tests in AGARS indicated that pinfish, Lagodon rhomboides, will avoid chlorine-produced oxidants at concentrations of 0.02-0.04 mg/ℓ (Fig. 26). The system is a prototype that can be enlarged by using more powerful lights and greater microprocessor memory capacity. In addition to several species of fishes, baseline data have also been obtained with blue crabs, Callinectes sapidus, and penaeid shrimp. The system could also be used to test thermal or salinity preferences.

TOXICANT INDUCED CHANGES IN CYCLIC BURROWING PATTERNS

The pink shrimp, Penaeus duorarum, is a species that is very sensitive to xenobiotics. Since no life-cycle toxicity test exists for penaeid shrimp, the only criterion of effect that has been used for hazard assessment is death. Pink shrimp normally remain buried in substrate during the day and emerge at night. Stress from both lethal and sublethal pesticide exposures disrupts this pattern and may result in the shrimp's continuous presence above the substrate. Such activity would increase predation and cycling of xenobiotics. To evaluate the effect of toxicant-induced disruptions in the cyclic burrowing pattern, an apparatus was constructed (Cripe 1979b) from a modified AGARS system. Two troughs were employed with sensors only in the upper level. Each trough was partially filled with sand and compartmentalized into four areas by plastic screen (Fig. 27). Shrimp were placed in each compartment on a 12L:12D photoperiod and monitored for six days. One trough was exposed to 2 ppb methyl parathion on days 3 and 4 of the test.

The results indicated variability in absolute activity level of a particular shrimp on different days as well as between shrimp. An activity index was calculated by dividing the mean of the hourly light beam interruptions for each dark period into the mean hourly counts for the succeeding light period. The index was lower for controls than for exposed shrimp. On

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a daily basis, there was significant difference in activity between days when toxicant was added and days when it was not (Fig. 28).

In conjunction with the development of trough systems, a device to detect potentially dangerous electrical currents in saltwater holding tanks was developed (Cripe & Stokes 1978).

SMALL SCALE MICROCOSMS

The usefulness of microcosms in evaluating fate and effect of various xenobiotics is well documented. Several of the tests developed under grant auspices were actually completed in laboratory microcosms. Bourquin et al. (1979) redescribed these, as well as three other types. A part of the grant effort was directed toward evaluating various aspects of the biology of selected species that could be used in microcosms.

POLYCHAETES

Pond culture of lugworms (D'Asaro, in press) was directly linked to development of techniques to use lugworms in small-scale systems. The results demonstrated rapid growth rates of lugworms over a 90-day period at densities of 60 or more worms per square meter when processed seagrass (Thalassia) was used as a food (Fig. 29). These data were used by Rubinstein (1979) to develop various toxicity tests in which lugworms were used as a primary component in the system.

White (1978) used microcosms to evaluate the impact of three predators (Neanthes succinea, Glycera americana, and Callinectes sapidus on lugworm prey. The blue crab, C. spaidus was the most effective predator especially on juvenile lugworms that do not burrow deeply.

Lasfargues (1981) examined the role of bacteria as a food source for lugworms in microcosms. Lugworms were allowed to consume composted seagrass; then comparisons were made between biomass of bacteria and yeast in the grass and feces. There was a significant reduction in the population of bacteria, but not in the population of yeast in the feces. There was also evidence of selective digestion of bacteria. A Gram-negative bacterium, isolated from composted seagrass, was cultured and used to enrich compost fed to worms. The enrichment caused a significant increase in growth.

Redig (1982) examined culture methods that could be used to rear Polydora ligni, a species that can be easily included in microcosms.

ISOPODS

Ligia exotica is a semiterrestrial isopod restricted to the immediate supralittoral zone, an area often heavily impacted by oil spills. Orientation and social behavior in L. exotica was evaluated by Farr (1978b). The tendency of L. exotica to aggregate and orient to environmental stimuli was examined in circular outdoor tanks or small aquaria.

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In the circular tanks, experimental animals released in the center of the arena were allowed to aggregate for 24 hours under clay saucers placed around the periphery of the arena. The resulting aggregations were not random, indicating that L. exotica actively seeks conspecifics. In tests in aquaria, L. exotica significantly selected shelters containing conspecifics or a shelter conditioned by previous occupancy by a conspecific. Other experiments demonstrated that distribution of L. exotica appears to be influenced initially by raised landmarks on the shoreline. Observations on social behavior were also reported.

Levy (1982) described the breeding habits and brood pouch development of Erichsonella attenuata, an isopod ideal for microcosm studies because it is easy to culture in closed systems, and a representative of marine grassflat communities.

MYSIDS

Plaia (1982) described the complete embryogenesis and organogenesis of Mysidopsis bahia, a species currently used as a standard organism in toxicity tests.

CONCLUSIONS

The toxic sediment bioassay system developed under this project shows promise of becoming a useful method in determining biological effects of potentially toxic sediments on representative estuarine organisms and benthic communities. It provides several distinct advantages over existing dredged material tests. Thus, it may be a suitable methodology for future inclusion in the testing manuals used to generate data in support of permitting programs such as S. 103 of The Marine Protection, Research and Sanctuaries Act of 1972.

The behavorial tests investigated demonstrate that measureable biological responses occur at contaminant levels below those acutely toxic. Further research is required, however, to achieve full understanding and interpretability of the information generated by these tests.

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REFERENCES

1. Ashton, Charles. 1981. The response of pinfish, Lagodon rhomboides, to cryptic and non-cryptic flounder, Paralichthys albigutta, an examination of the value of cryptic shading to a predator. Bull. Mar. Sci. (in press).

2. Bourquin, A.W., R.L. Garnas, P.H. Pritchard, F.G. Wilkes, C.R. Cripe, and N.I. Rubinstein. 1979. Interdependent microcosms for the assessment of pollutants in the marine environment. Intern. J. Environ. Studies., 13:131-140.

3. Cripe, C.R. 1979a. An automated device (AGARS) for studying avoidance of pollutant gradients by aquatic organisms. J. Fish. Res. Bd. Can., 35(1):11-16.

4. Cripe, C.R. 1979b. An automated apparatus to record toxicant-induced changes in cyclic burrowing patterns of pink shrimp (Penaeus duorarum). Research Review, September 1979, pp 26-27.

5. Cripe, C.R. and C.E. Ashton. 1979. Predator-prey bioassay system. Research Review, September 1979, pp 28.

6. Cripe, C.R., and B.E. Stokes. 1978. A device to detect potentially dangerous electrical currents in saltwater holding tanks. Progressive Fish. Cult., 40(2):74-75.

7. D'Asaro, C.N. 1982. Pond culture of the lugworm, Arenicola cristata, as a source of bait. Aquaculture (in press).

8. Farr, J.A. 1977. Impairment of antipredator behavior in Palaemonetes pugio by exposure to sublethal doses of parathion. Trans. Amer. Fish. Soc., 106(3):287-290.

9. Farr, J.A. 1978a. The effect of methyl parathion on predator preference for two estuarine prey species. Trans. Amer. Fish. Soc., 107(1):87-91.

10. Farr, J.A. 1978b. Orientation and social behavior in the supralittoral isopod Ligia exotica. Bull. Mar. Sci., 28(4):659-666.

11. Garnas, R.L., C.N. D'Asaro, N.I. Rubinstein, and R.A. Dime. 1977. The fate of methyl parathion in a marine benthic microcosm. Paper #44 in Pesticide Chemistry Division, 173rd ACS meeting, New Orleans, Louisana, March 20-25, 1977.

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12. Lasfargues, J.E. 1981. Evaluation of bacteria as a food resource for Arenicola cristata. Masters thesis, University of West Florida.

13. Levy, I.C. 1982. Breeding habits and ontogeny of Erichsonella attenuata reared in the laboratory. Masters thesis, University of West Florida.

14. Plaia, W.C. 1982. Embryogenesis and organogenesis of Mysidopsis bahia. Masters thesis, University of West Florida.

15. Redig, M.X. 1982. Optimal temperature-haline conditions for development of Polydora ligni. Masters thesis, Florida State University.

16. Rubinstein, N.I. 1978. Effect of sodium pentachlorophenate on the feeding activity of the lugworm, Arenicola cristata. In: K.R. Rao, ed., Chemistry, Pharmacology and Environmental Toxicology of Pentachlorophenol, Plenum Press, New York, pp. 175-179.

17. Rubinstein, N.I. 1979. A benthic bioassay using time-lapse photography to measure effects of toxicants on feeding behavior of lugworms. In: W.B. Vernberg et al., Marine Pollution: Functional Responses. Academic Press, New York. pp. 341-355.

18. Rubinstein, N.I., R.A. Dime, and C.E. Ashton. 1980a. Chrysene uptake and depuration by the lugworm (Arenicola cristata). In: N. Richards, Proceedings of a Symposium on Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment, Academic Press, New York.

19. Rubinstein, N.I., F.G. Wilkes, C.N. D'Asaro, and C. Sommers. 1980b. Effects of contaminated sediments on representative estuarine organisms and developing benthic communities. In: Robert Baker, editor, Contaminants and Sediments, Vol. 1., Ann Arbor Sciences. pp. 445-461.

20. Rubinstein, N.I,. R.A. Rigby, and C.N. D'Asaro. 1980c. The acute and sublethal effects of whole used drilling fluids on representative organisms. In: Proceedings and Symposium on Research on Environmental Fate and Effects of Drilling Fluids and Cuttings, Vol. 4. Am. Petrol. Institute, Washington, D.C. pp. 828-846.

21. White, C.B. 1978. Predation on cultured lugworms (Arenicola cristata). Masters thesis, University of West Florida.

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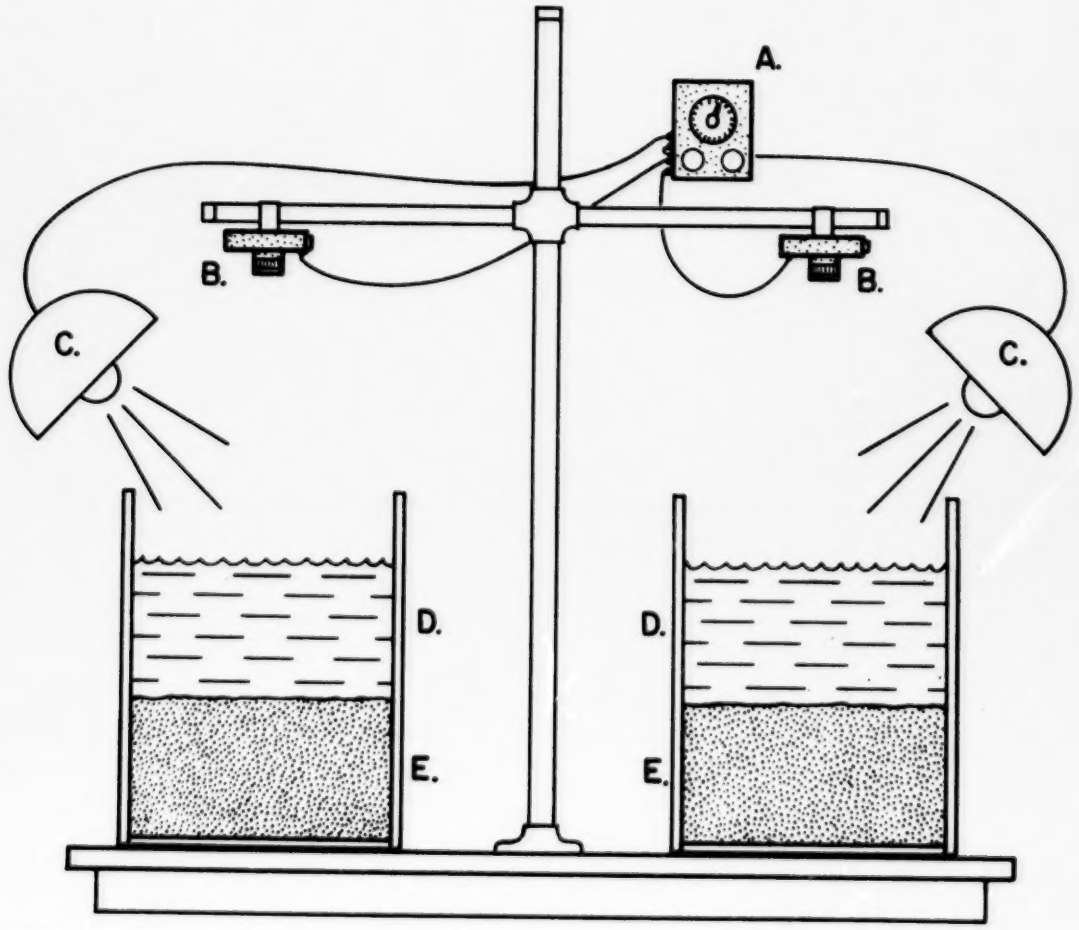

Fig. 1. Photo-Bioassay System (A - 24-hour timer; B - 35 mm camera with automatic advance; D, E - aquaria with 25 cm of sand and 75 ℓ of seawater).

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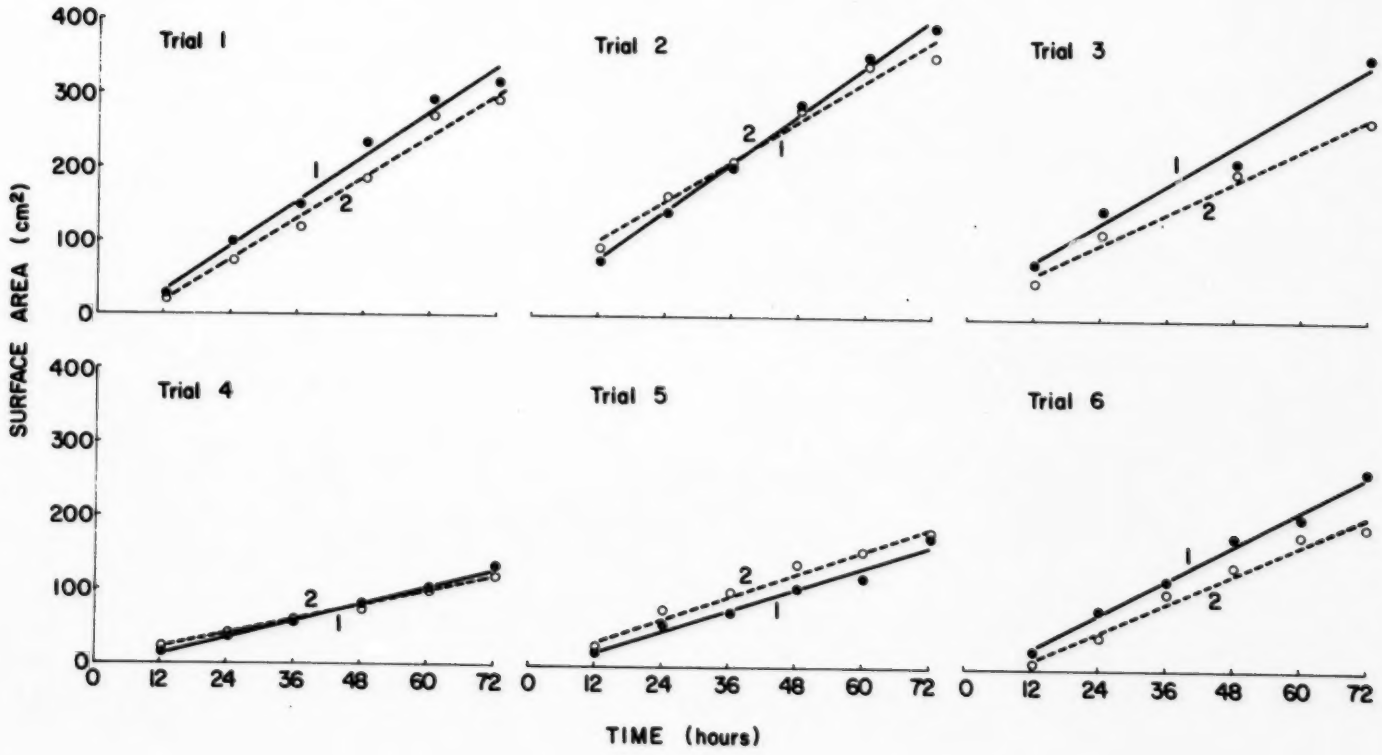

Fig. 2. Comparison of the rates of sediment turned under by groups of similar size. A different group of lugworms was used for the six replicate tests.

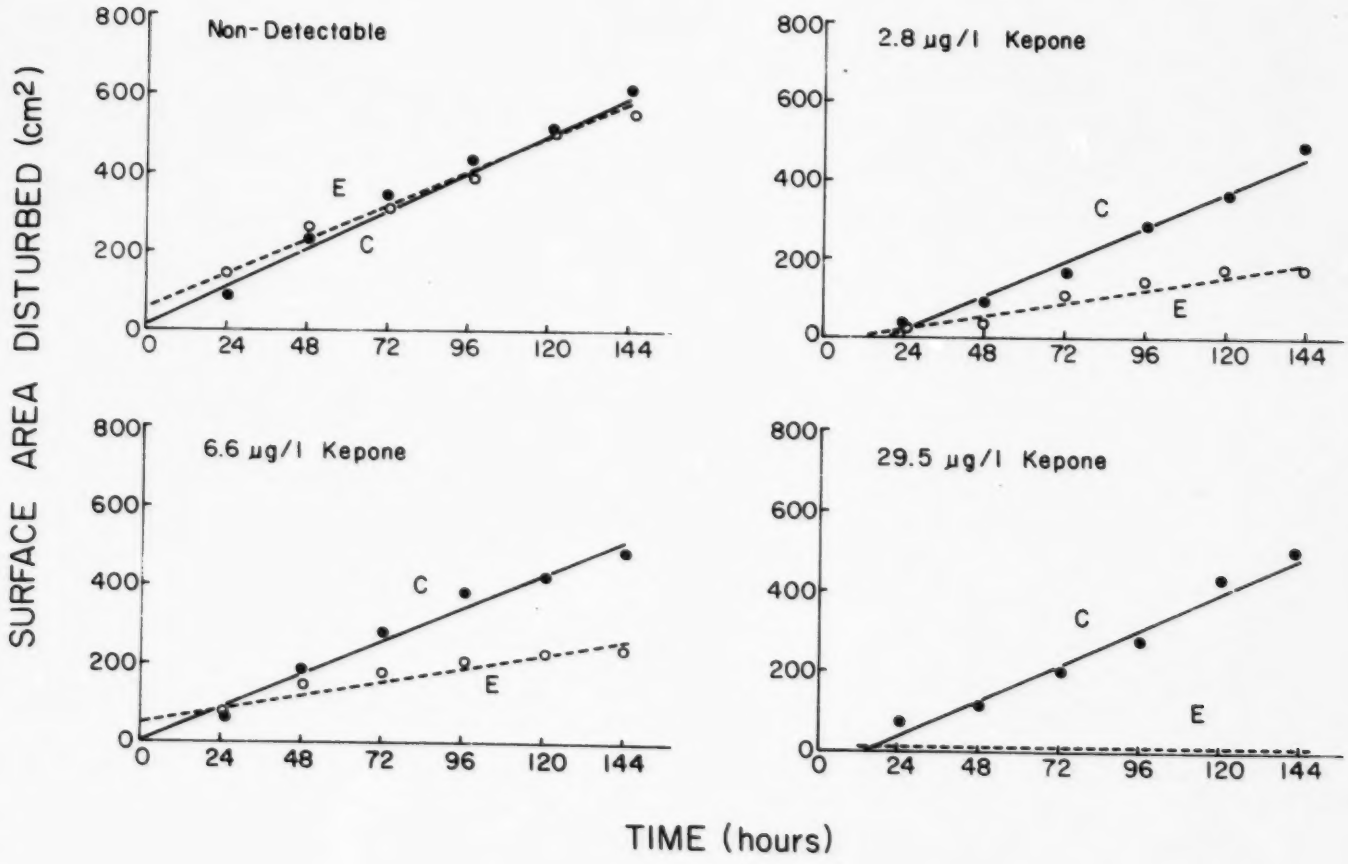

Fig. 3. Comparison of the rates of sediment turned under by lugworms (C - control; E - experimental group exposed to Kepone). Each group consisted of six lugworms.

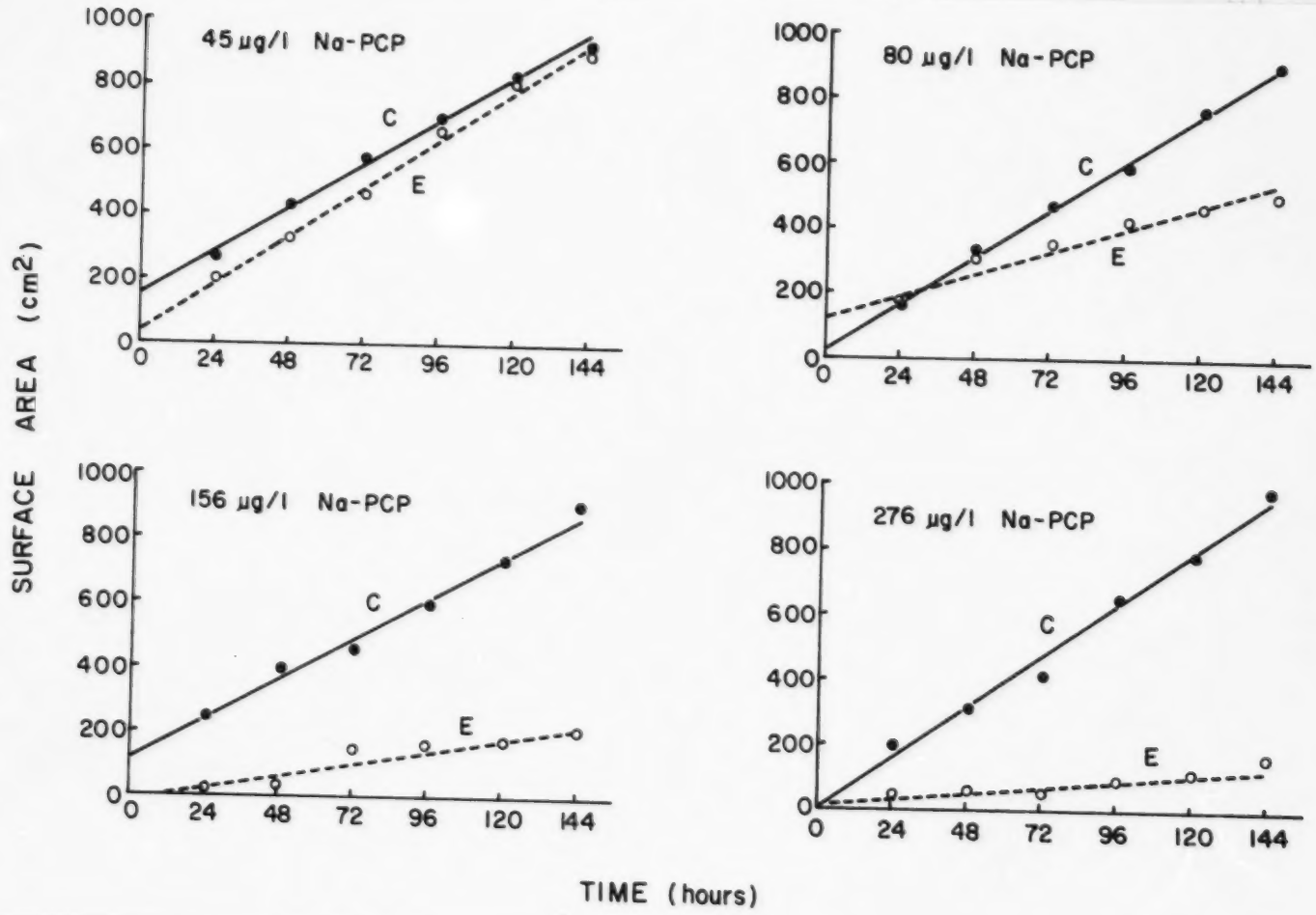

Fig. 4. Comparison of the rates of sediment turned under by the lugworm, Arenicola cristata (C - controls; E - experiment group exposed to sodium pentachlorophenate). Each group consisted of six lugworms.

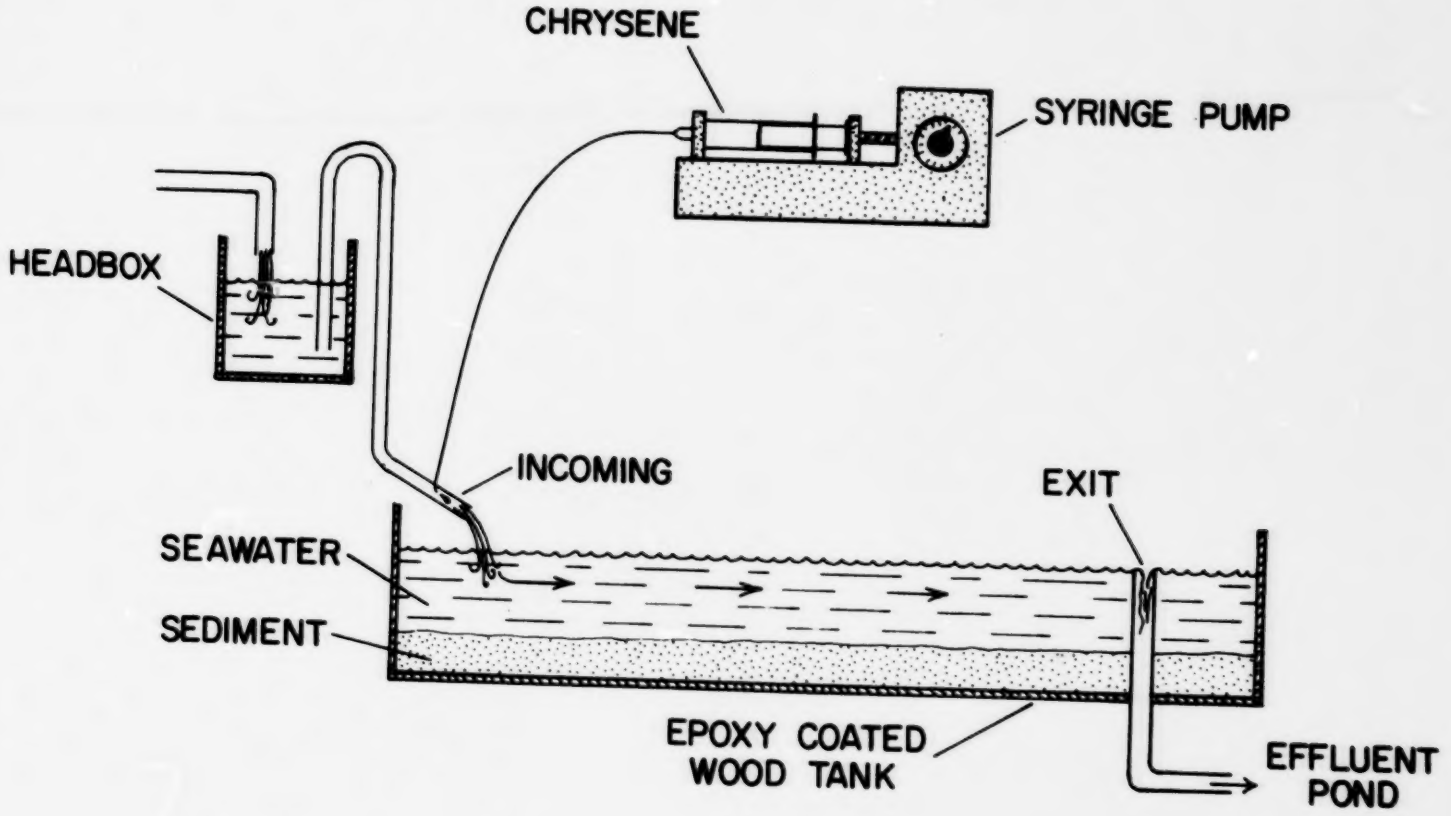

Fig. 5. One tank in the exposure system for chrysene.

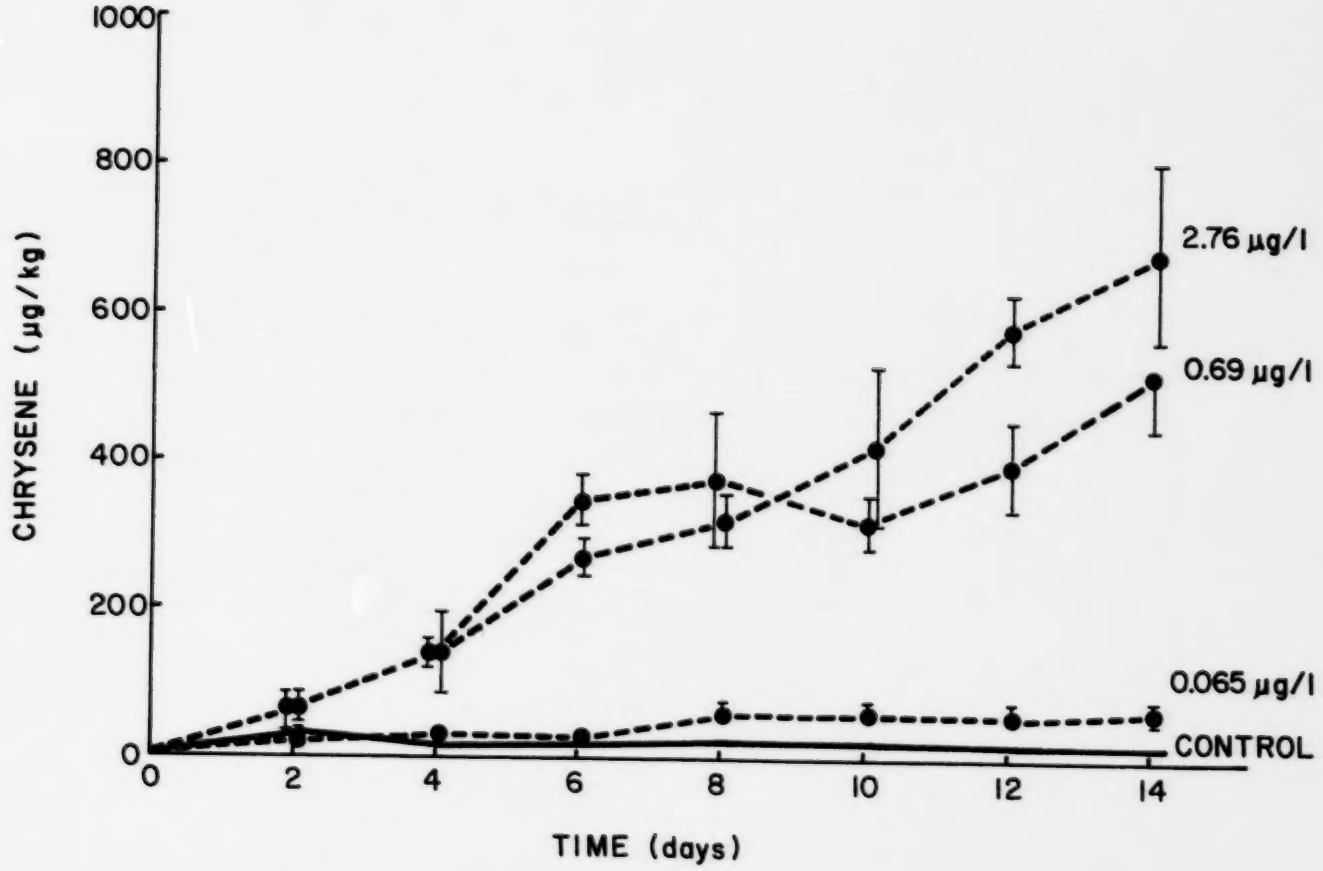

Fig. 6. Accumulation of chrysene by lugworms. Each data point is based on average accumulation by five worms.

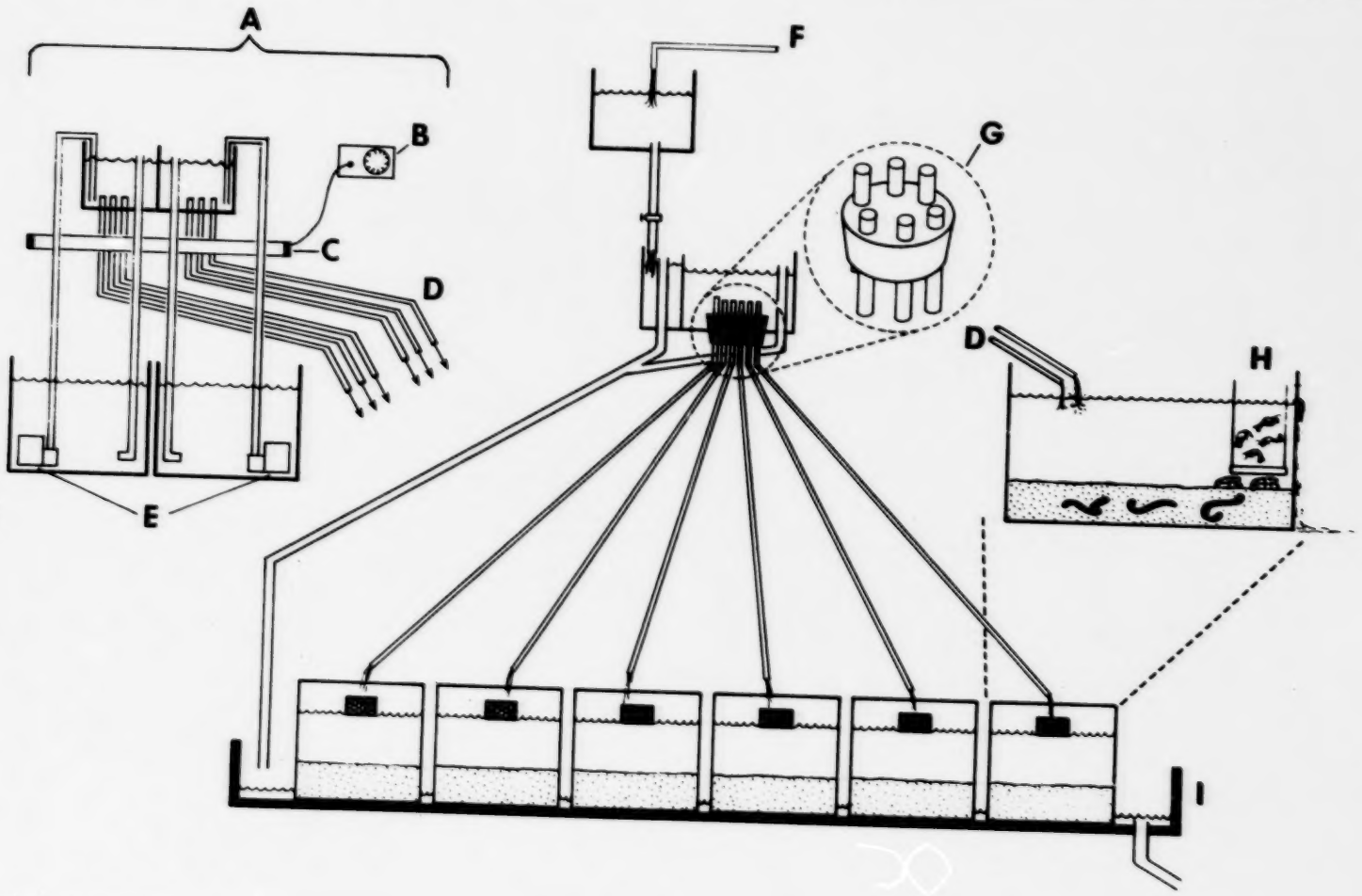

Fig. 7. Exposure system. A - suspended-sediment dosing apparatus; B - timer; C - crimping bar and solenoids; D - delivery tubes; E - submersible pumps to recirculate suspended sediment to the delivery box (not labeled); F - seawater headbox; G - splitter box stand pipes; H - mysids, oysters and lugworms in exposure tanks on a water table; I - exposure tanks on a water table.

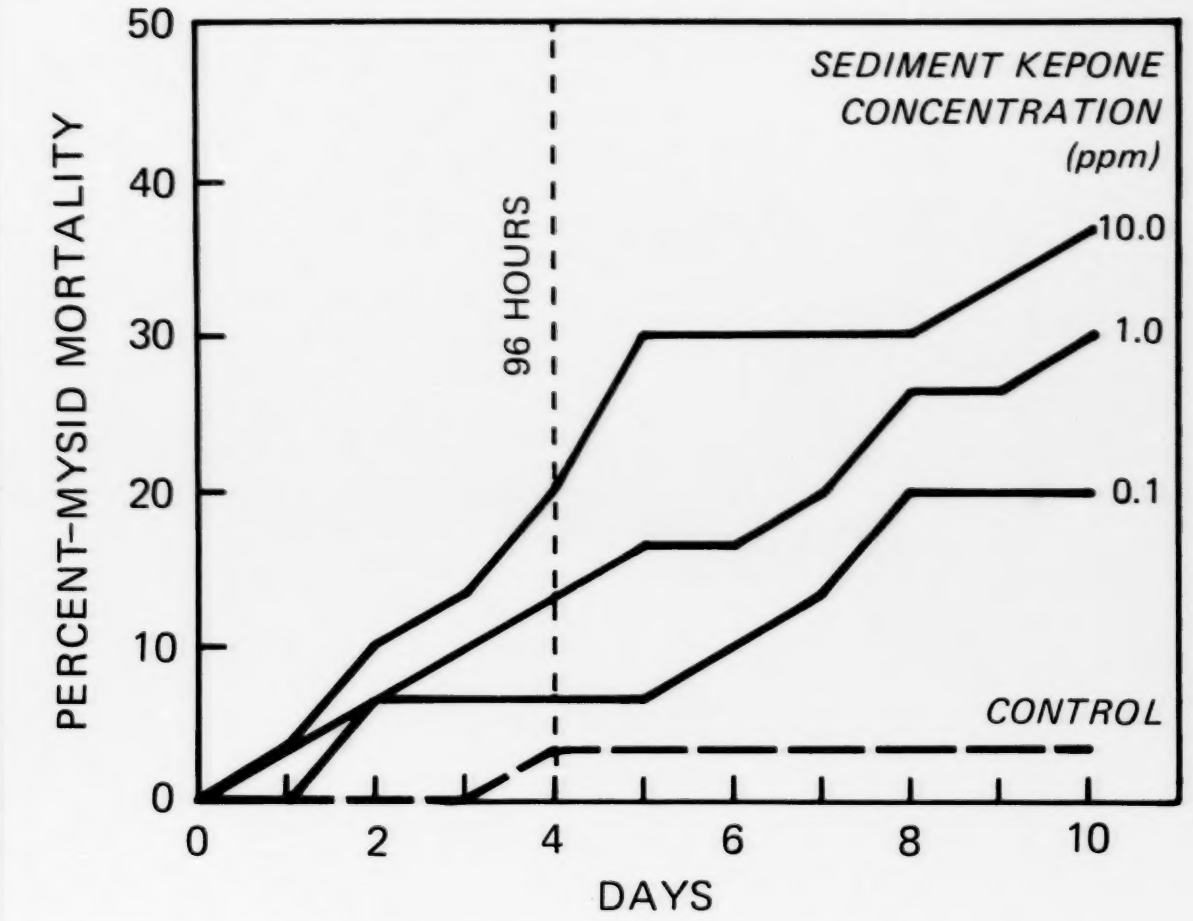

Fig. 8. Mortality of mysids exposed to control and Kepone-sorbed sediments for 10 days.

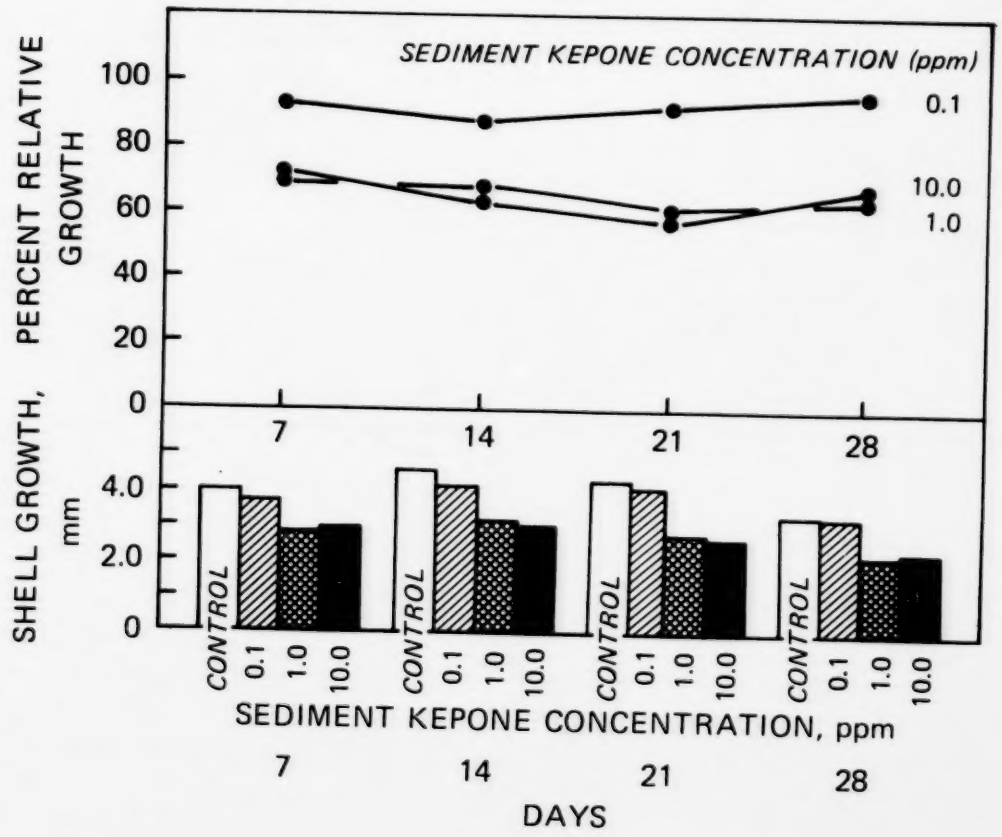

Fig. 9. Effect of Kepone-sorbed sediments on oyster shell deposition (percentage growth is relative to controls).

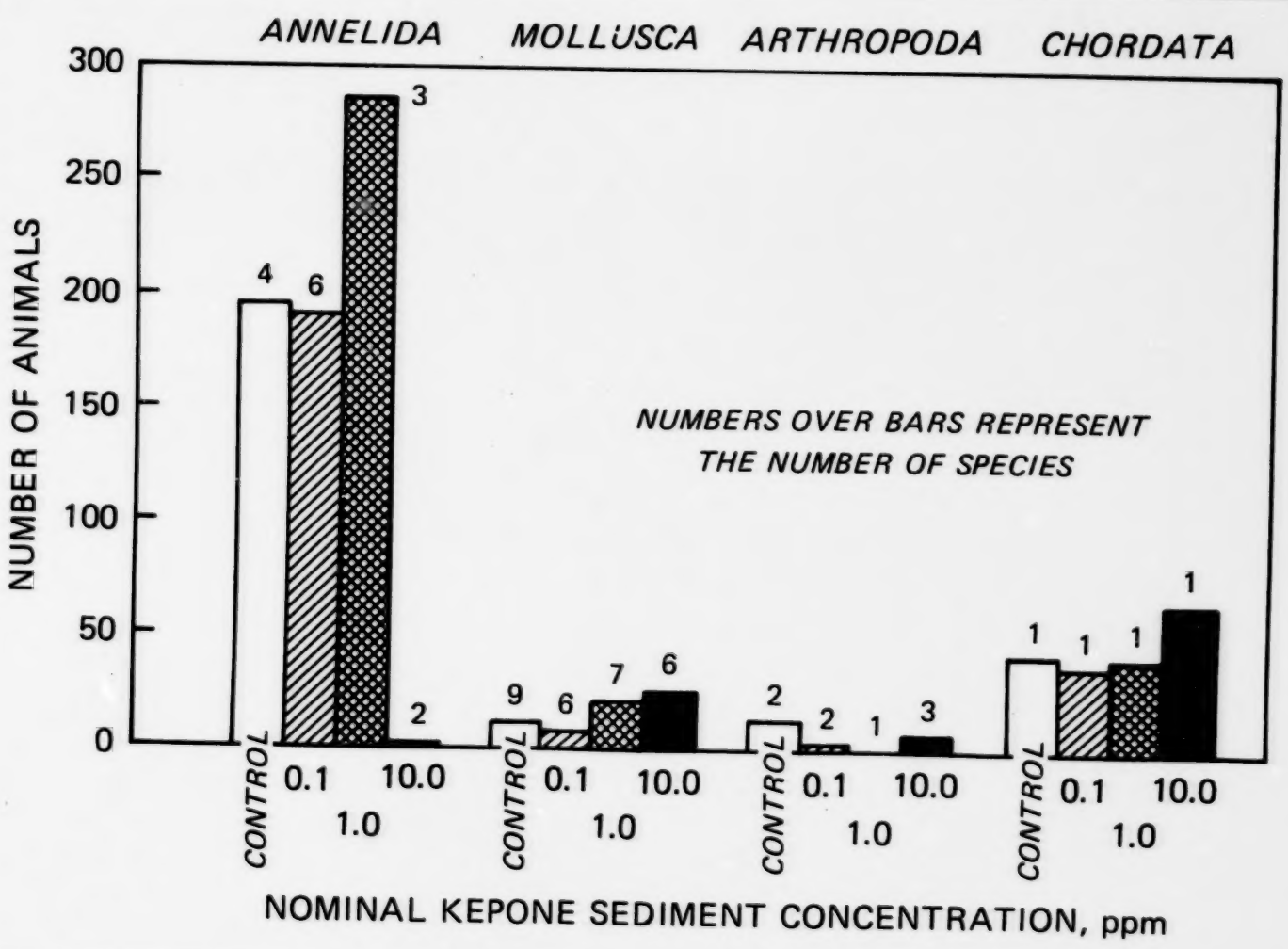

Fig. 10. Number of individuals and species collected from exposed and control aquaria after 28 days.

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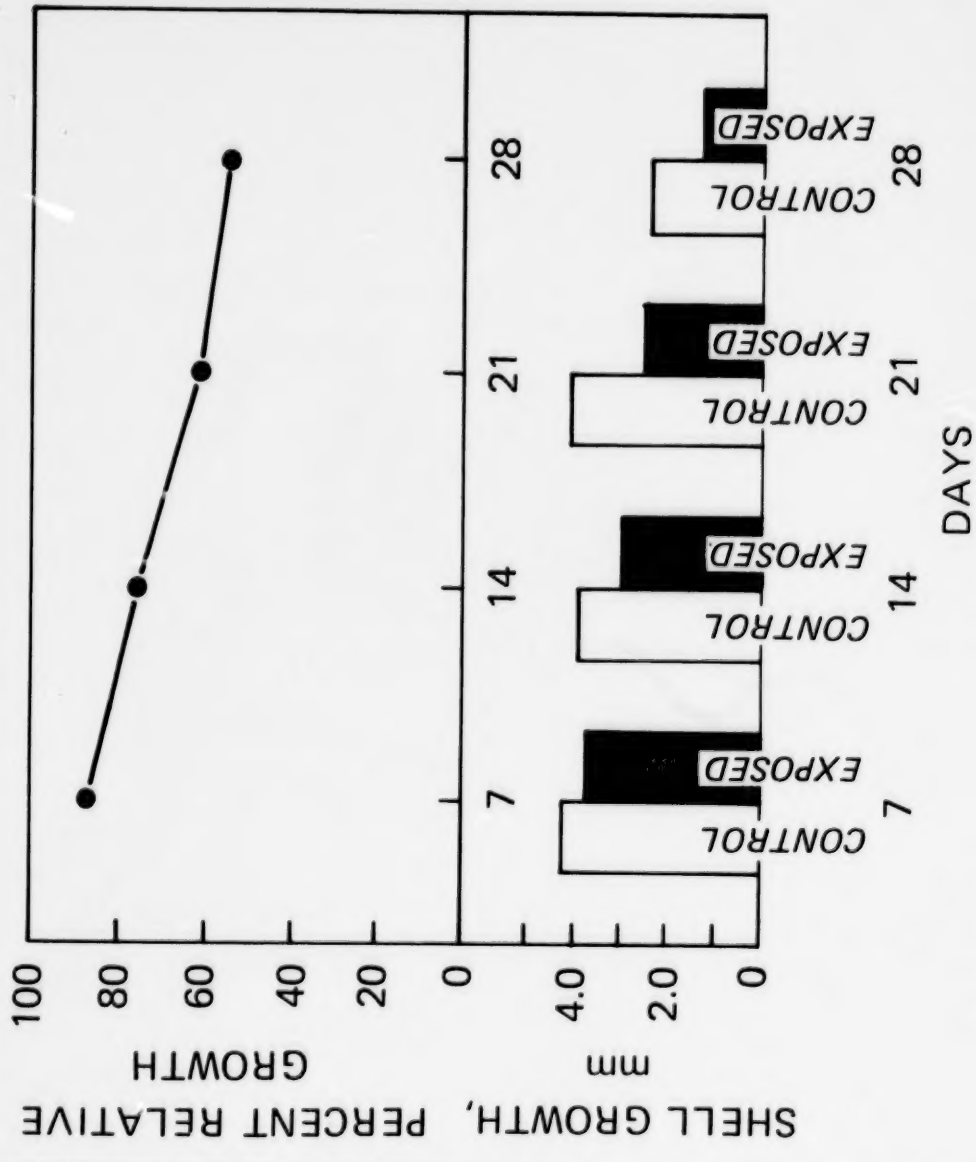

Fig. 11. Effect of James River sediment on oyster shell deposition.

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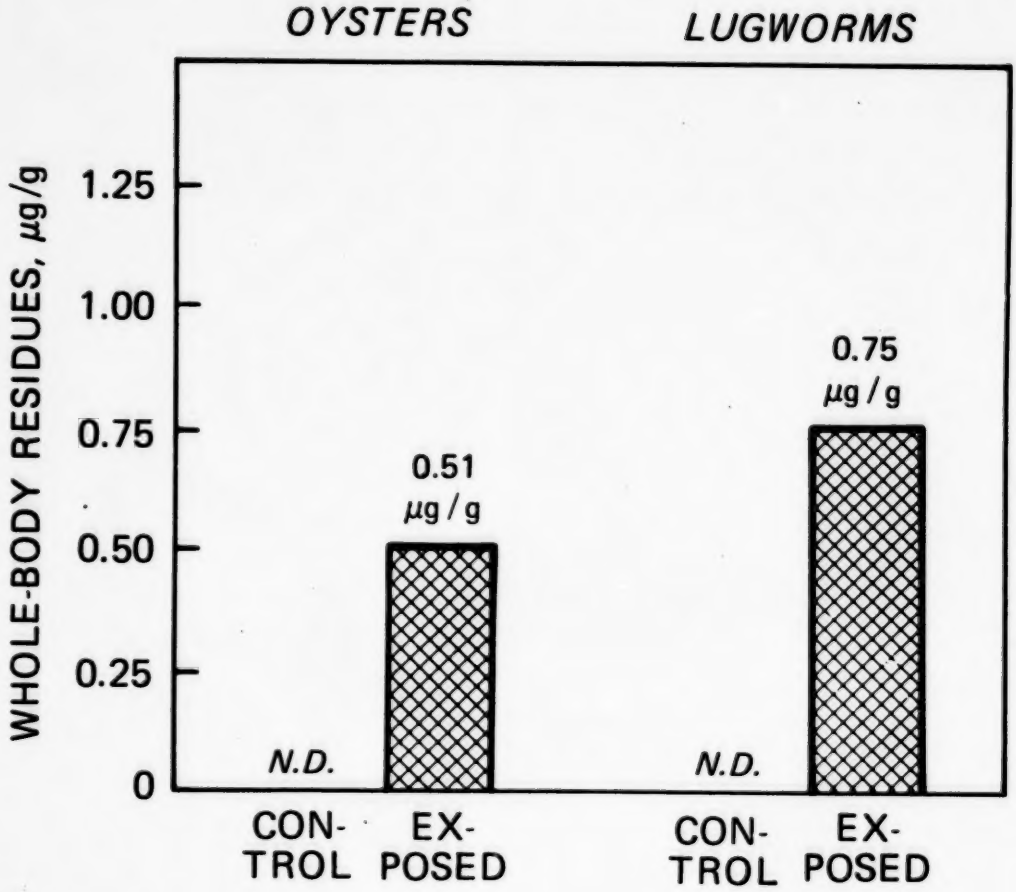

N.D., NON-DETECTABLE (<0.02 µg/g).

Fig. 12. Kepone residues in oysters and lugworms following 28 days of exposure to James River sediments.

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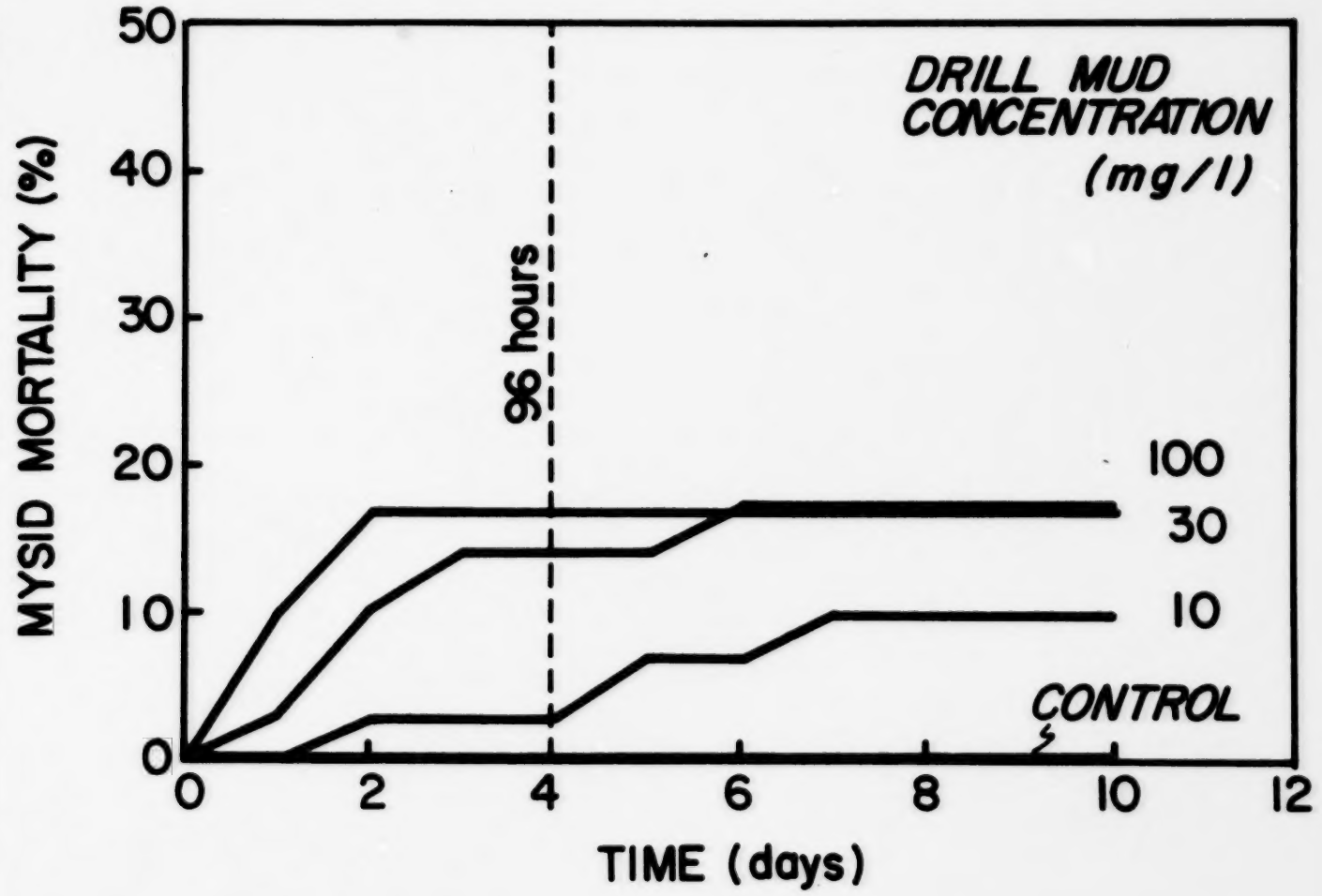

Fig. 13. Mortality of mysids exposed to control and three concentrations of drilling muds for 10 days.

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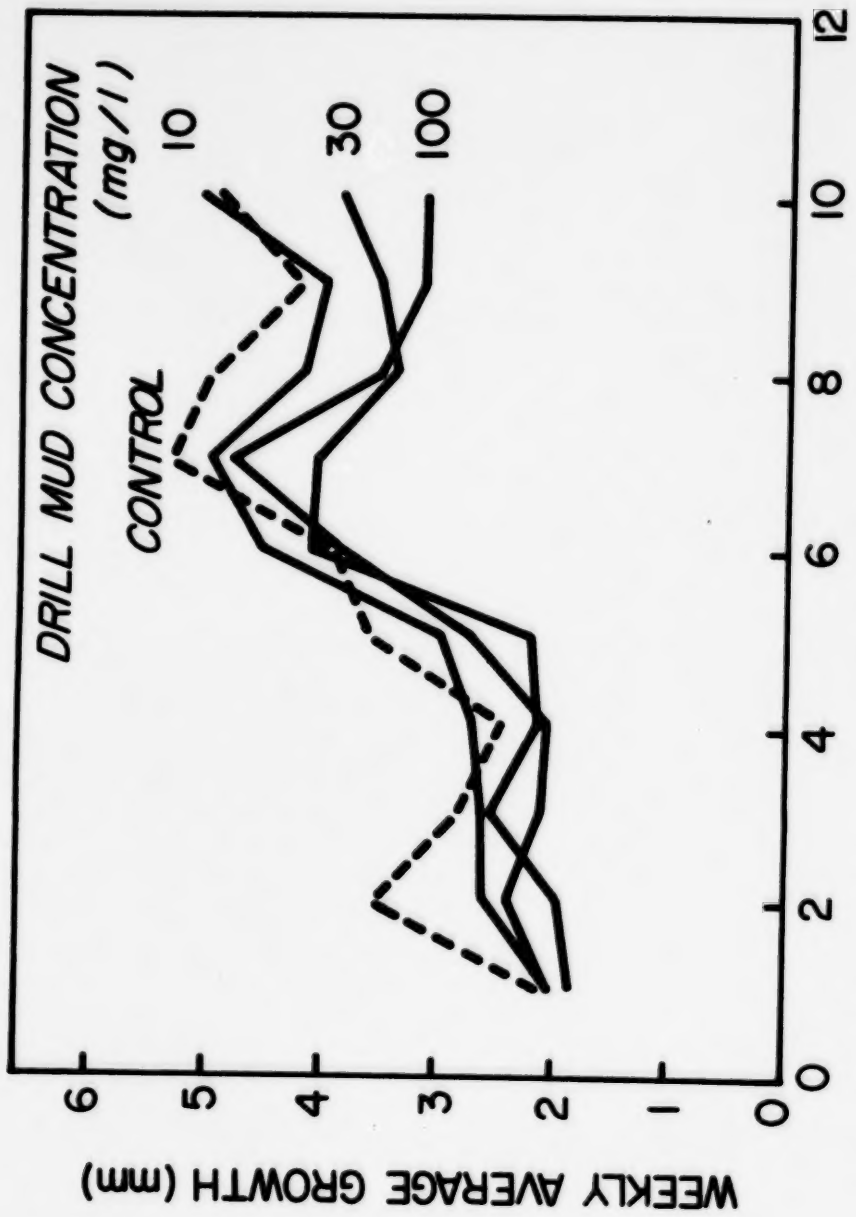

Fig. 14. Weekly average oyster growth (N=15).

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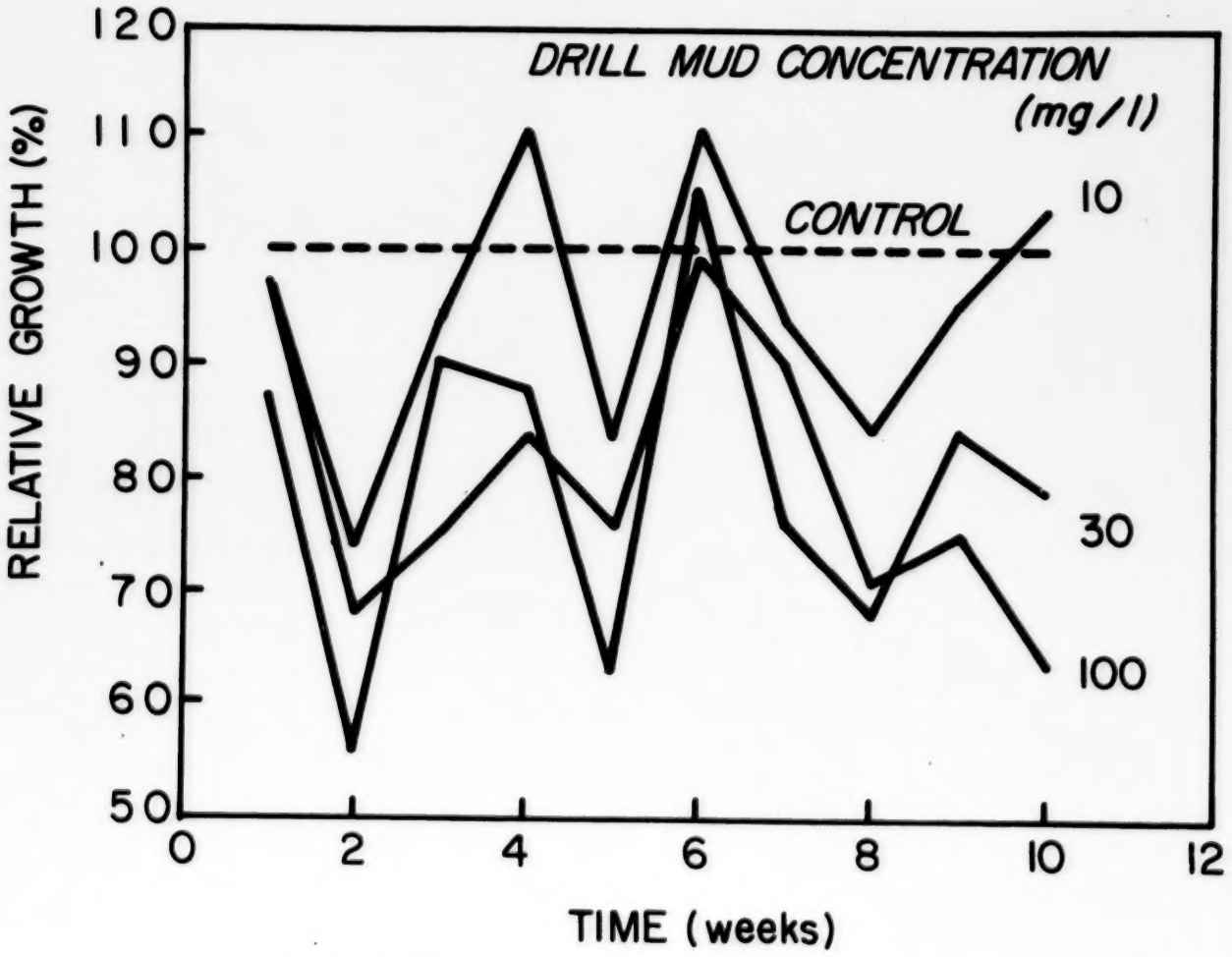

Fig. 15. Oyster shell deposition expressed as a percentage of growth of control oysters.

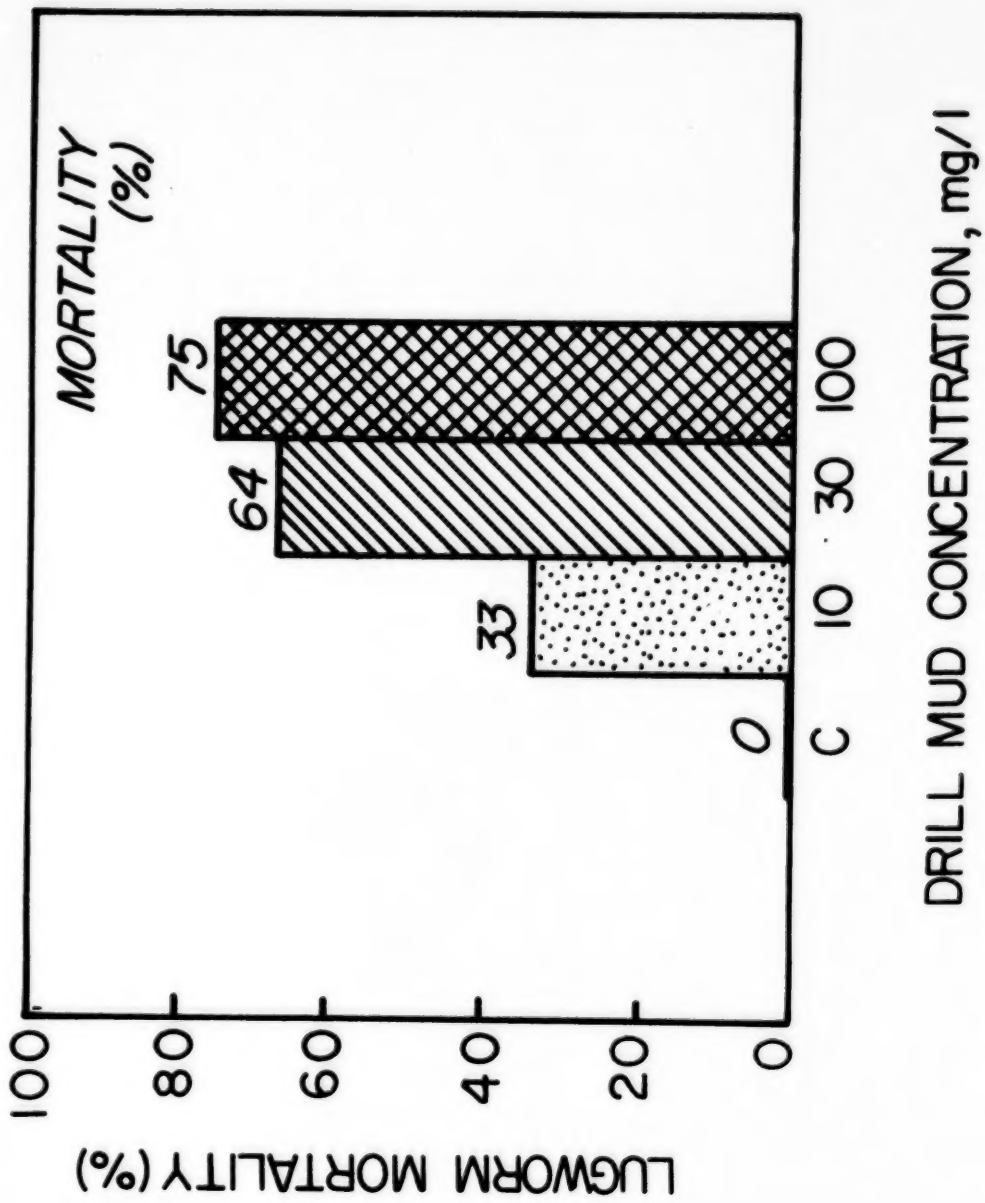

Fig. 16. Mortality of lugworms in control and exposed aquaria.

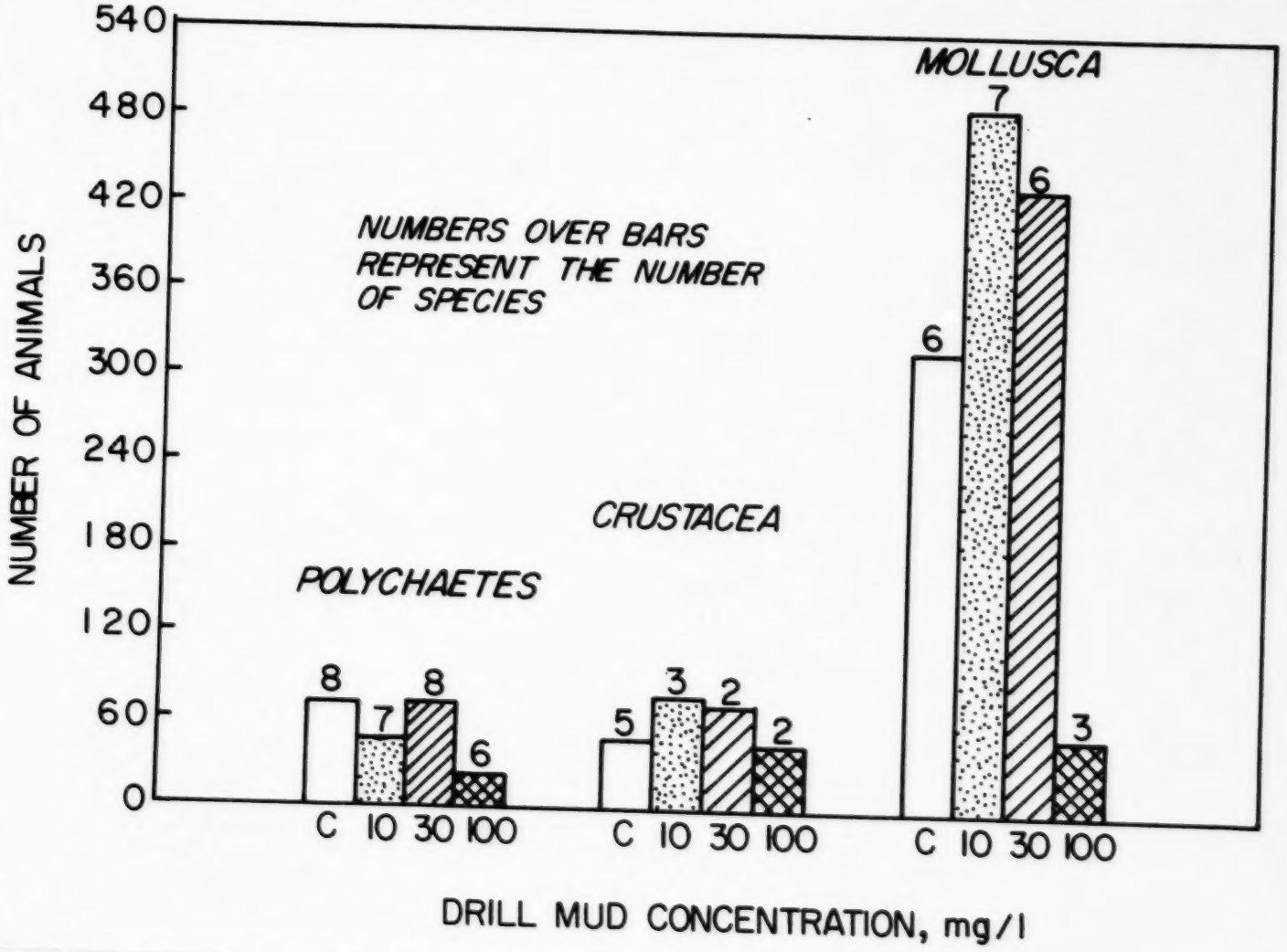

Fig. 17. Number of individuals and species collected from exposed and control aquaria after 100 days.

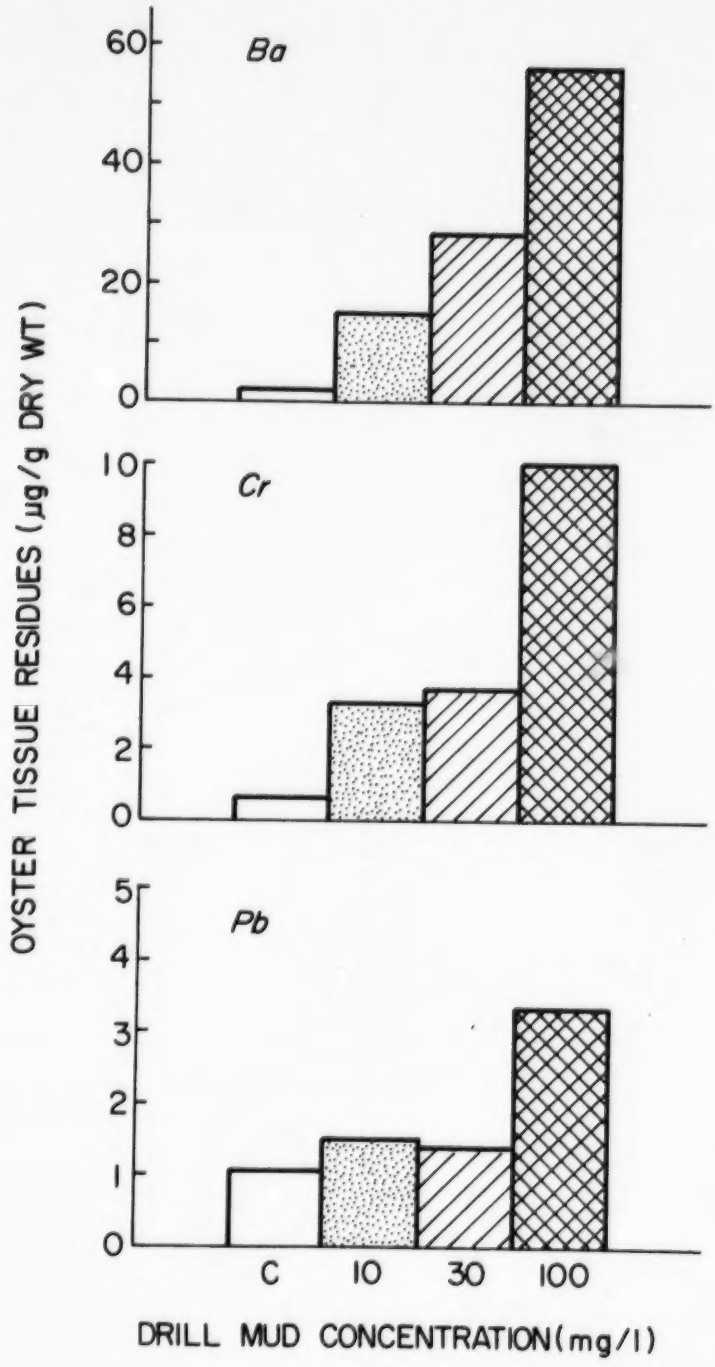

Fig. 18. Ba, Cr, and Pb concentrations in oysters exposed to control and three concentrations of drilling muds for 100 days.

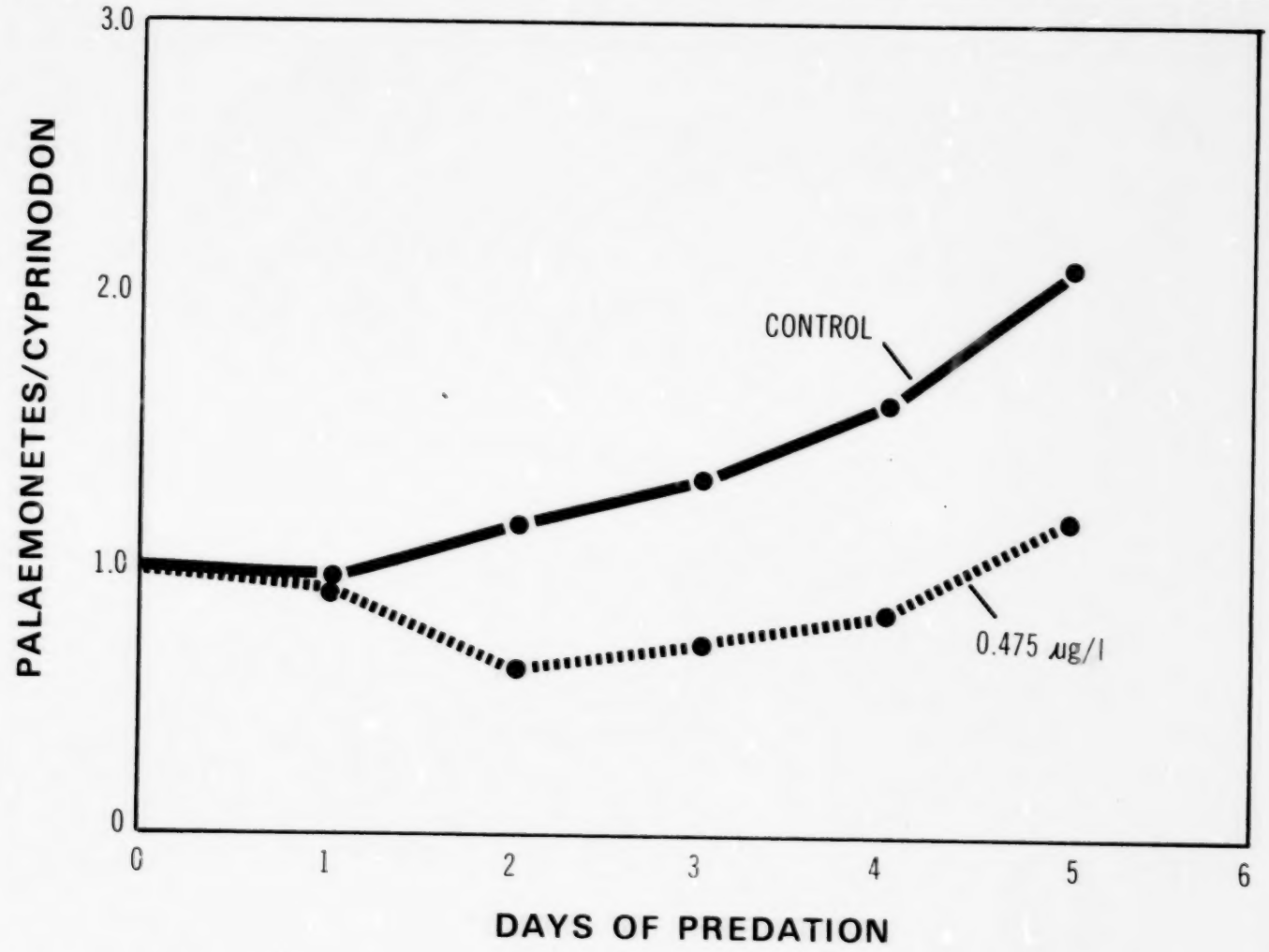

Fig. 19. The ratio of Palaemonetes pugio to Cyprinodon variegatus that survived after each of five days of predation by Fundulus grandis in control and 0.475 mg/ℓ methyl parathion-exposed aquaria. The lower ratio in the exposed aquaria indicated greater predation on P. pugio.

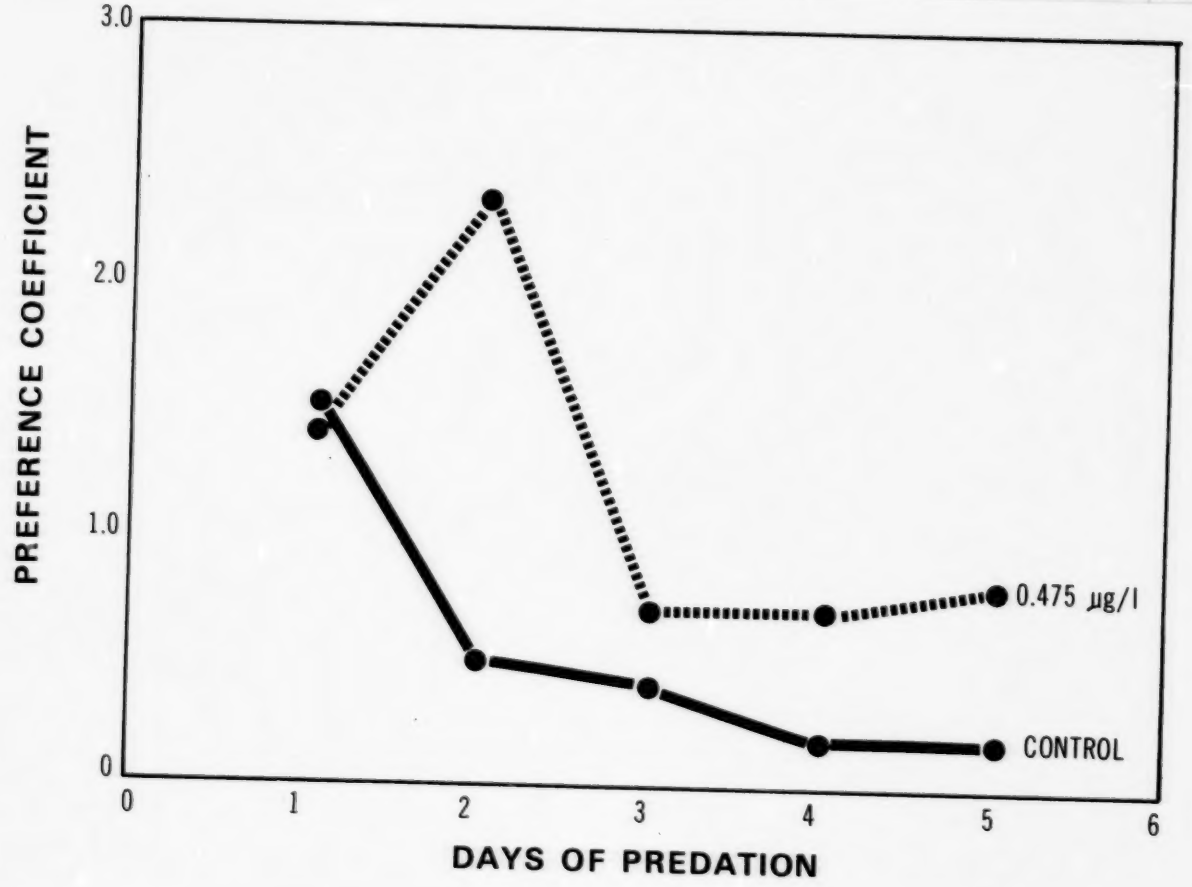

Fig. 20. Preference coefficients for F. grandis predation on P. pugio and juvenile C. variegatus during five test days in control and 0.475 mg/ℓ methyl parathion-exposed aquaria. Higher coefficients indicate greater predation on P. pugio. See Farr (1978) for method of calculation.

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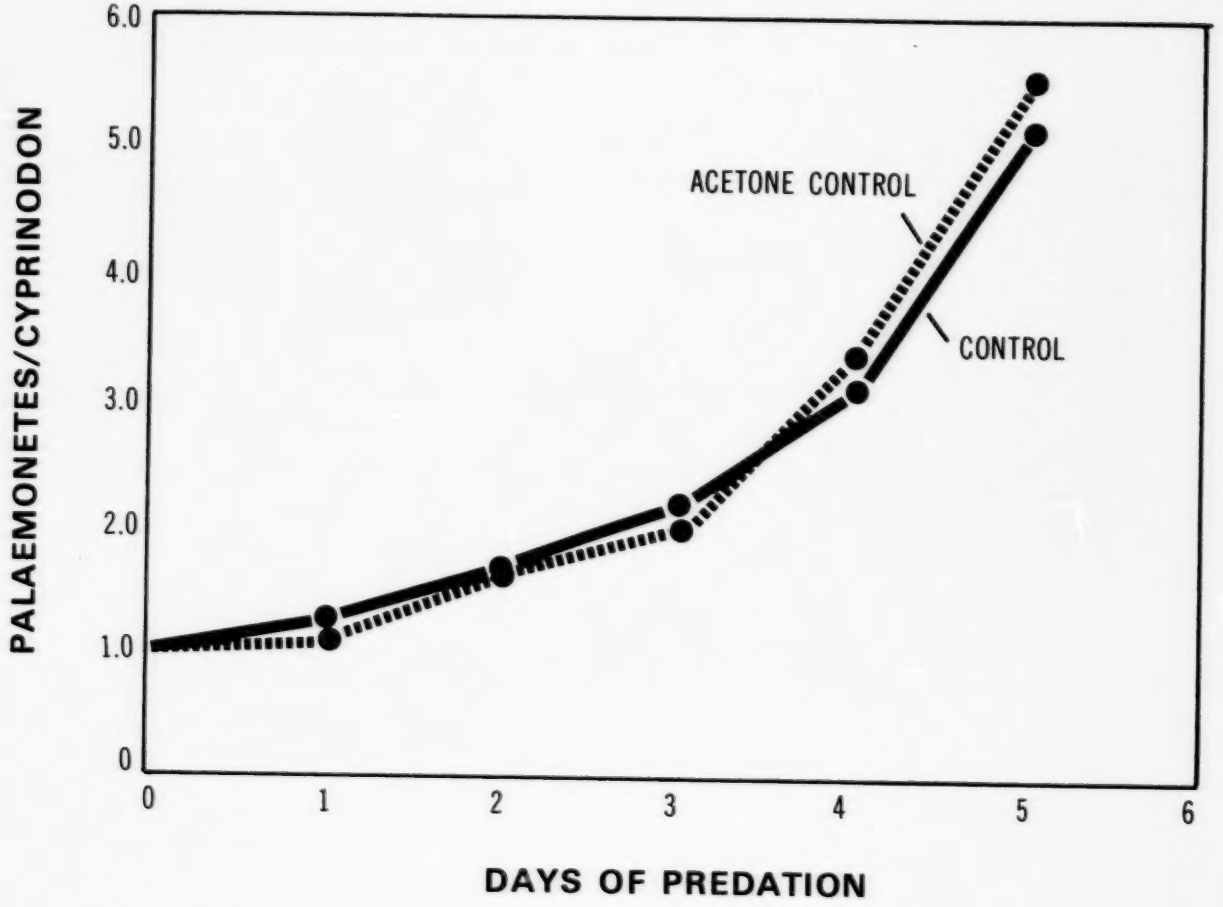

Fig. 21. The ratio of P. pugio to C. variegatus that survived after each of five days of predation by F. grandis in control aquaria and in acetone-control aquaria.

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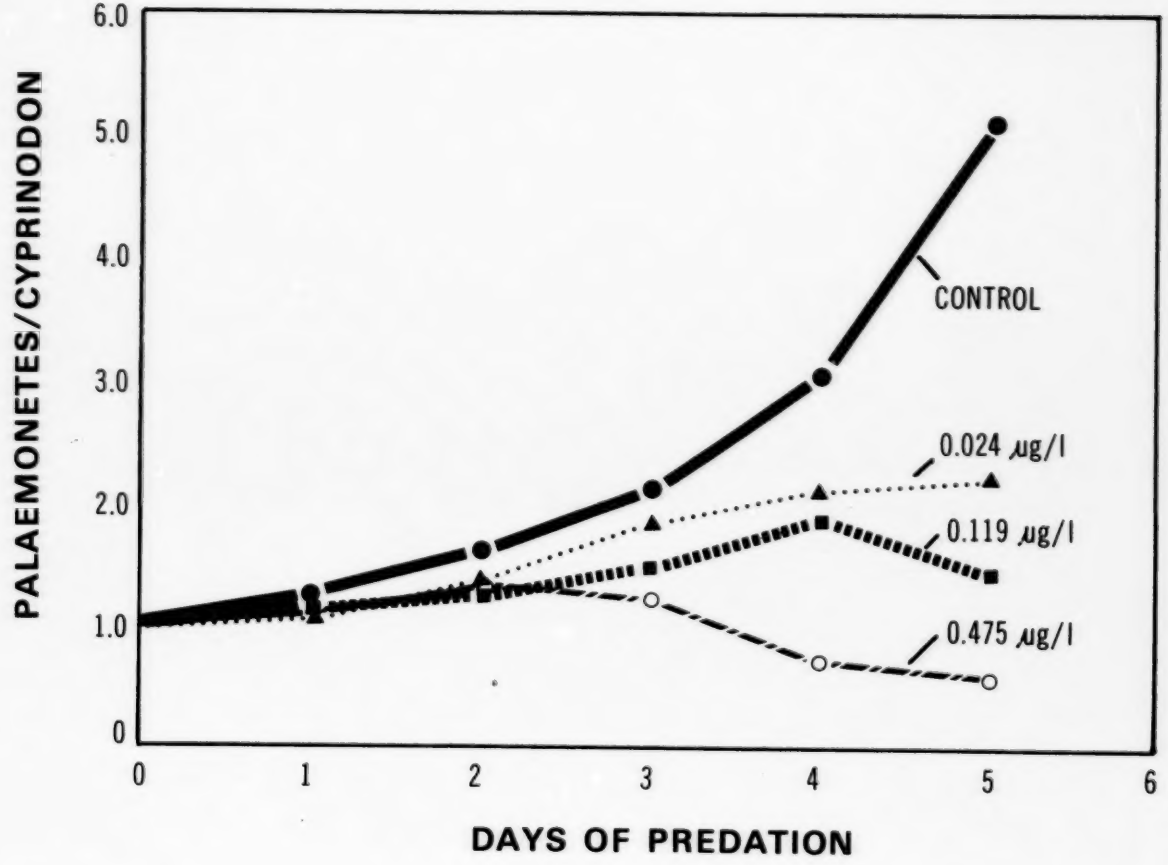

Fig. 22. The ratio of P. pugio to C. variegatus that survived after each of five days of predation by F. grandis in control aquaria and in concentrations of methyl parathion.

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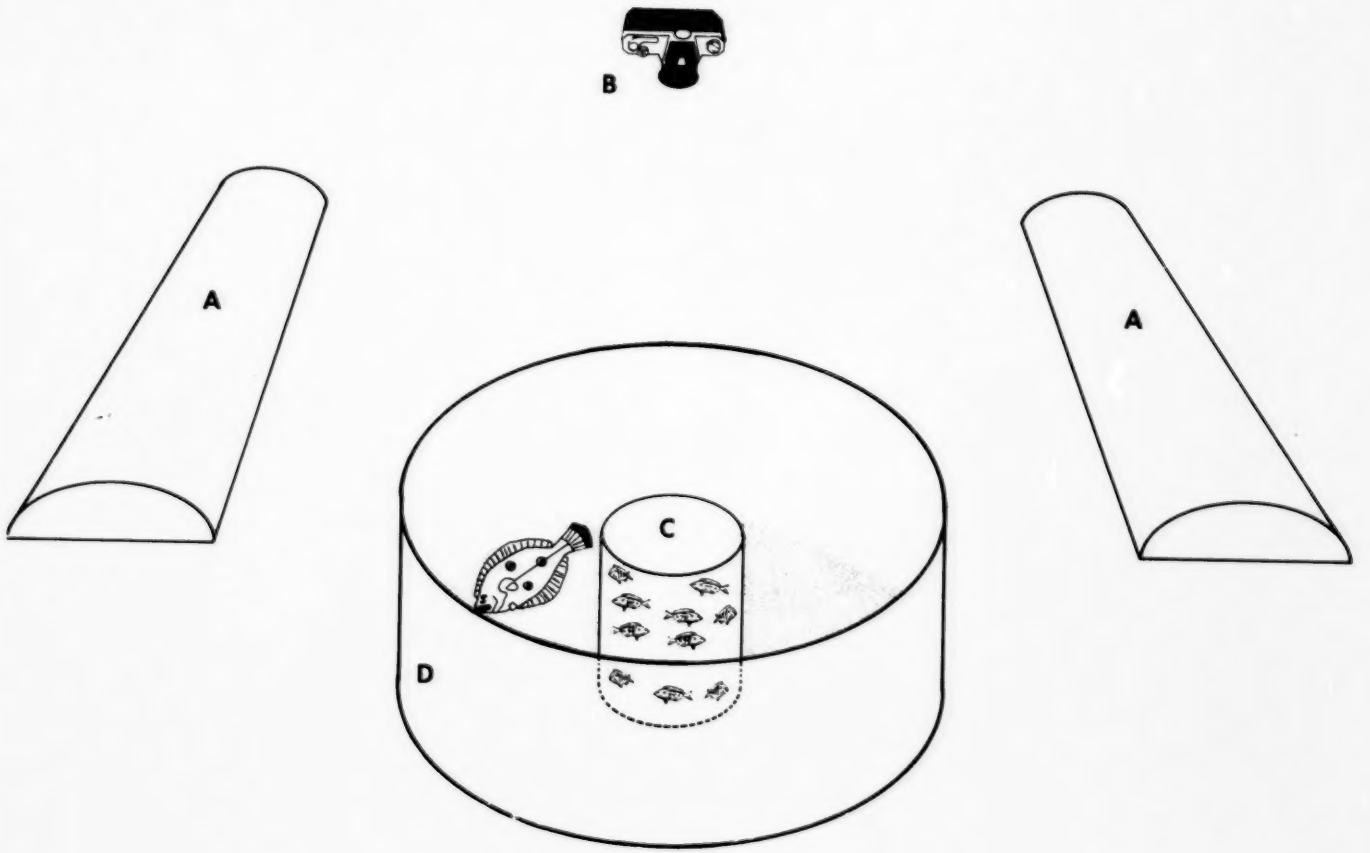

Fig. 23. Test apparatus used to observe pinfish reactions to flounder models (A - fluorescent lights; B - automatic advance camera; C - release chamber; D - circular test tank).

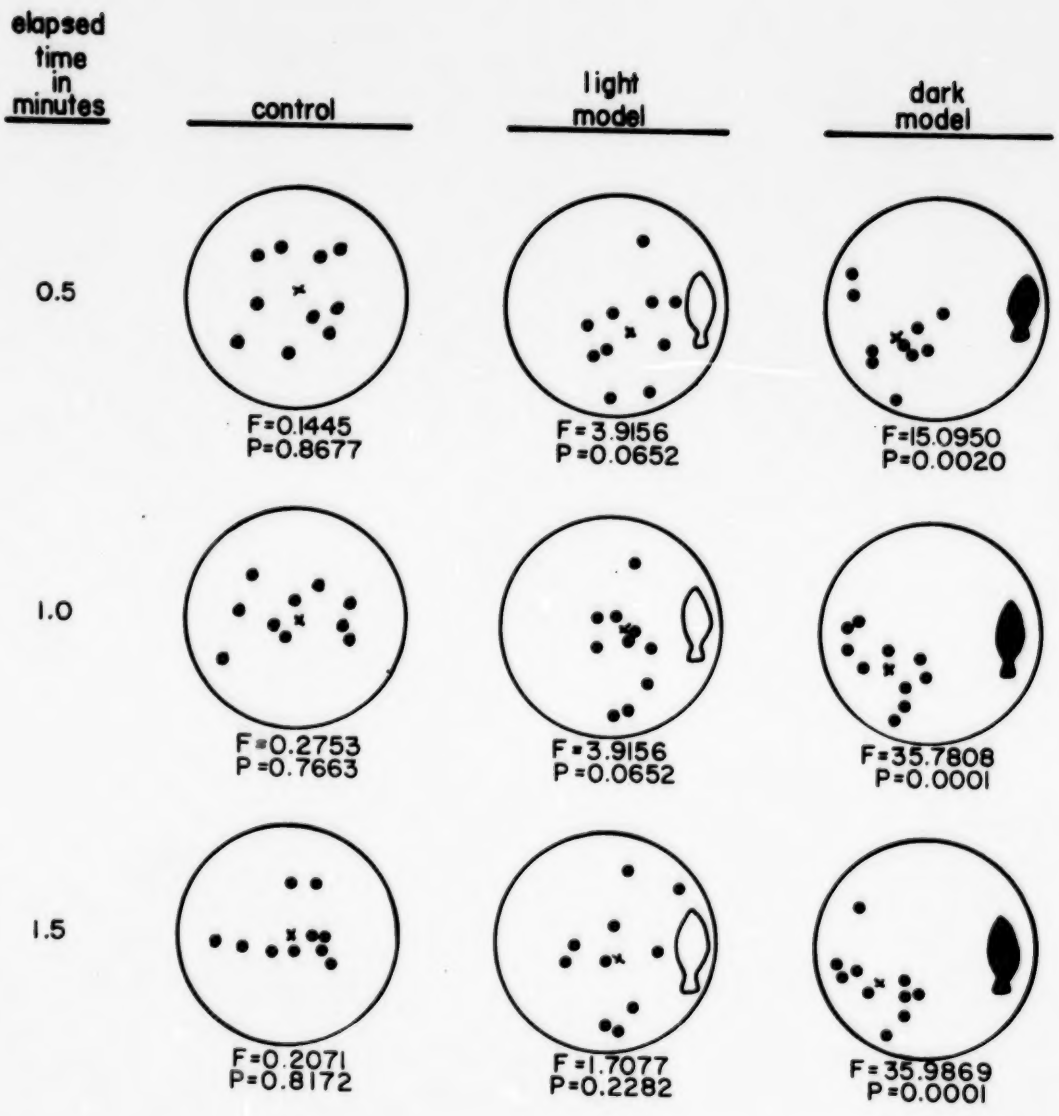

Fig. 24. Diagrams of pinfish positions for combined trials 1-10 for each time-interval of control, light flounder model, and dark flounder model. Each point represents the mean position of 10 pinfish for each trial, and X represents the mean of all 10 points. The F values shown below the diagram were calculated by Hotelling's one-sample test. P values correspond to the probability at the given F value.

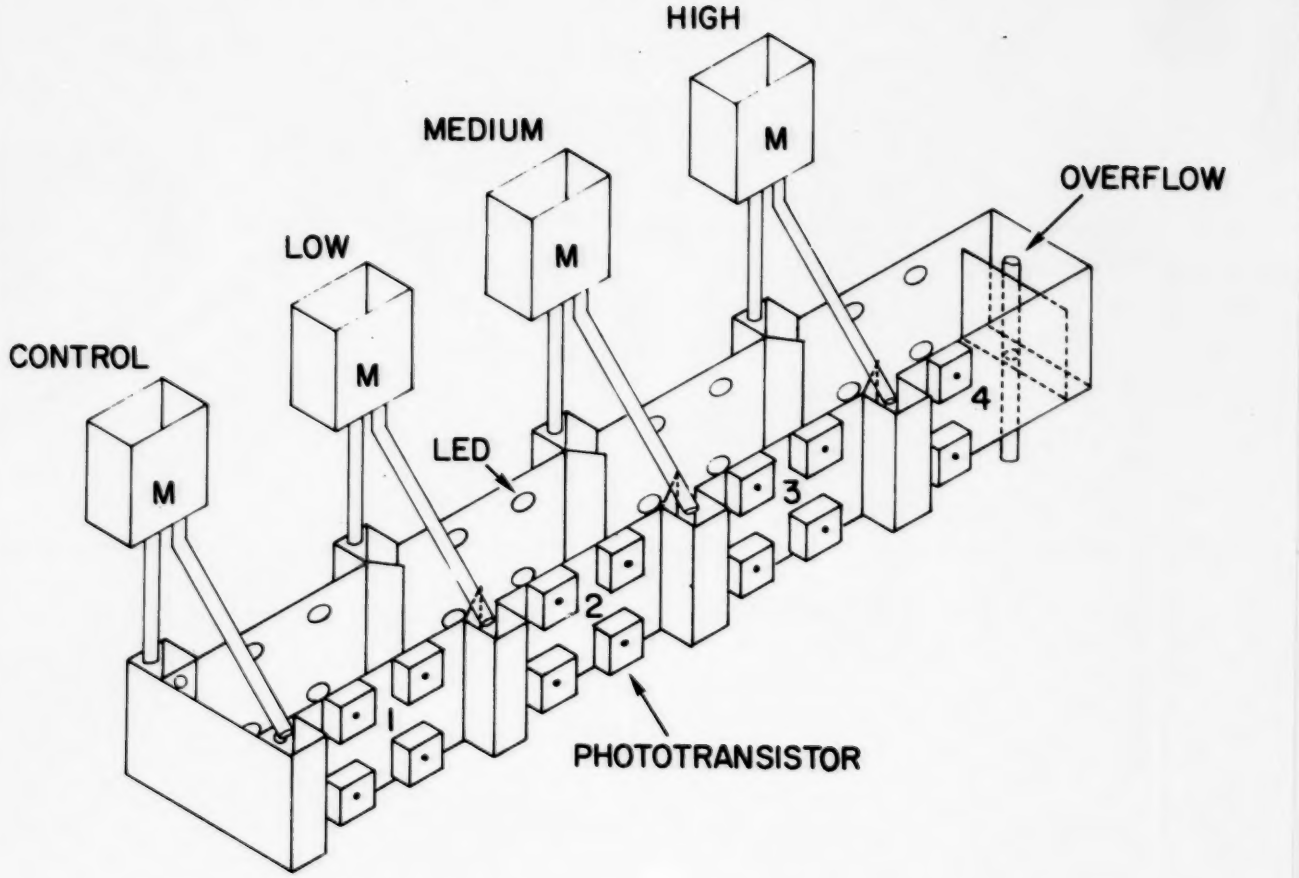

Fig. 25. Diagram of AGARS trough. Trough is 125 x 17 x 15 cm high and constructed of 6-mm clear acrylic plastic. "M" denotes mixing boxes where 1.5 ℓ clean water min and test compounds or carriers are combined. The flow is divided evenly between pairs of small chambers on each side of the trough. Water exits from the chambers through a row of 7-mm holes. Water flow maintains a gradient of a control zone and three increasing toxicant concentrations (areas 1-4). Organism position can be monitored in both the upper and lower half of each area by pairs of infrared light emitting diodes and photo-transistors.

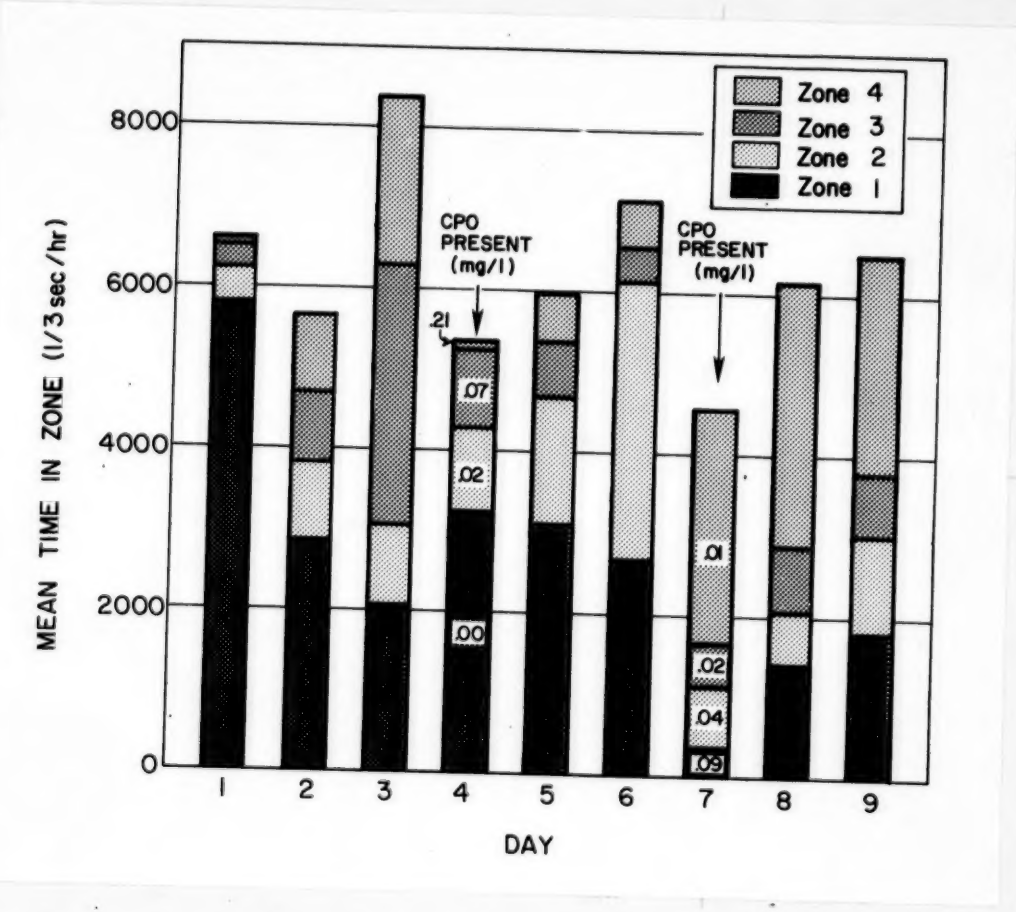

Fig. 26. Results of a 9-day AGARS test with a group of 4 pinfish. Mean of 24-hourly totals of time spent in each of 4 areas of the trough versus elapsed time of test. Chlorine-produced oxidants were present on days 4 and 7 only.

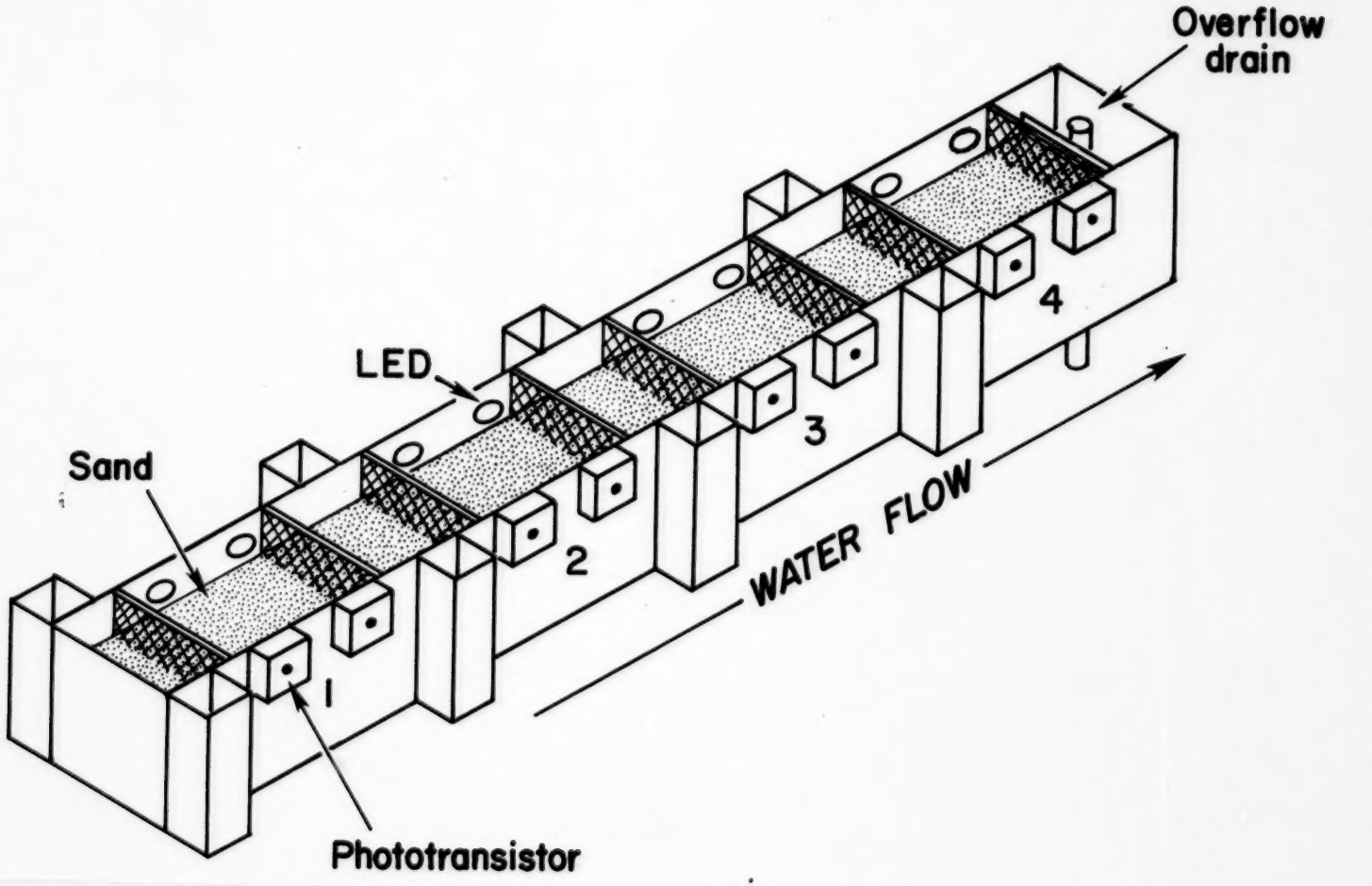

Fig. 27. Diagram of one of two replicate troughs used to study pink shrimp behavior. The trough was modified from that shown in Figure 26 in that it is partitioned with barriers of acrylic plastic and plastic screen and contains sand. The presence of each shrimp above the sand is monitored by two pairs of photo-transistors and infrared light-emitting diodes.

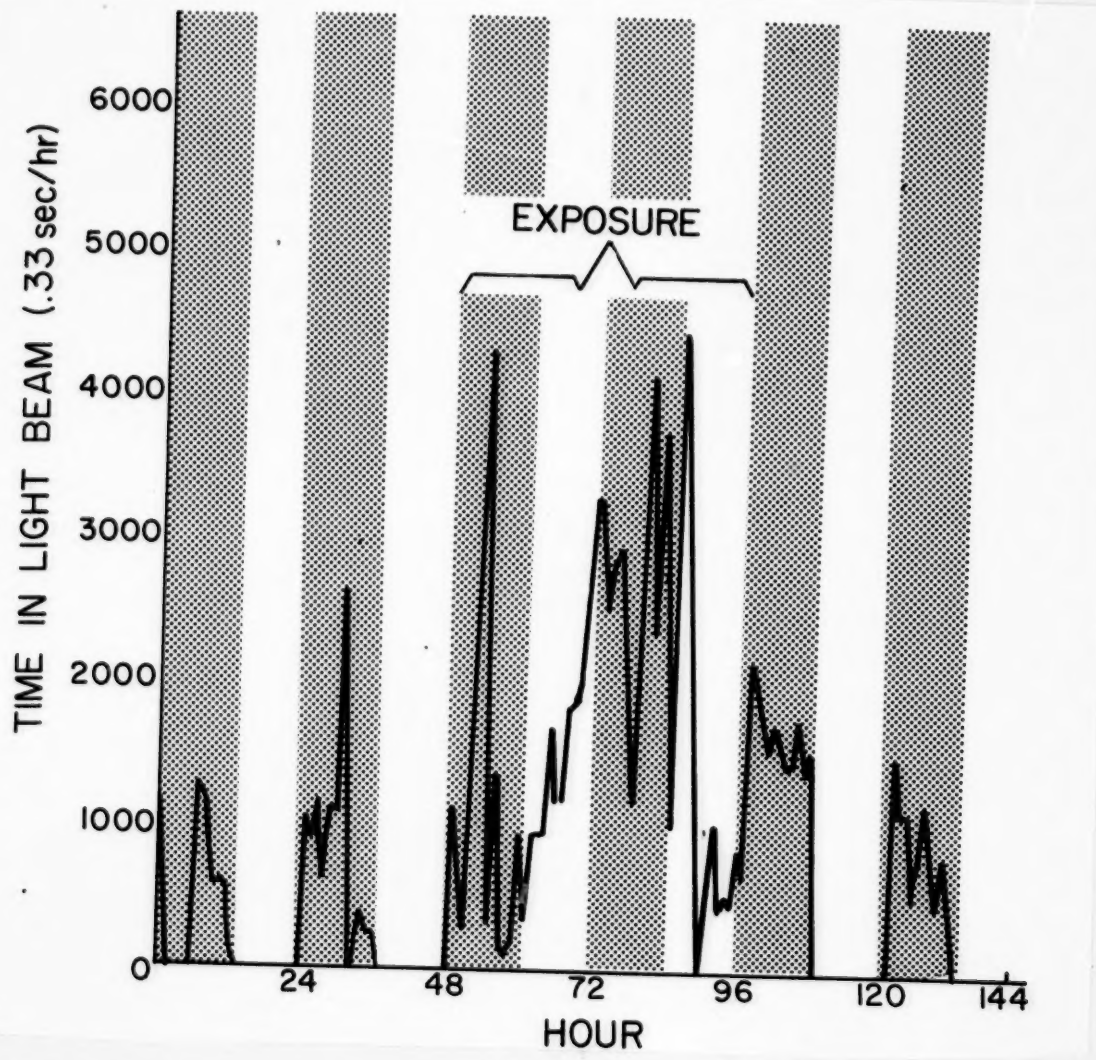

Fig. 28. Example of an activity graph indicating time in light beams (0.33 sec/h) during each hour of the 6-day test. On days 3 and 4, shrimp were exposed to 2.0 ppb methyl parathion and carrier. Light and dark bars indicate photo-period.

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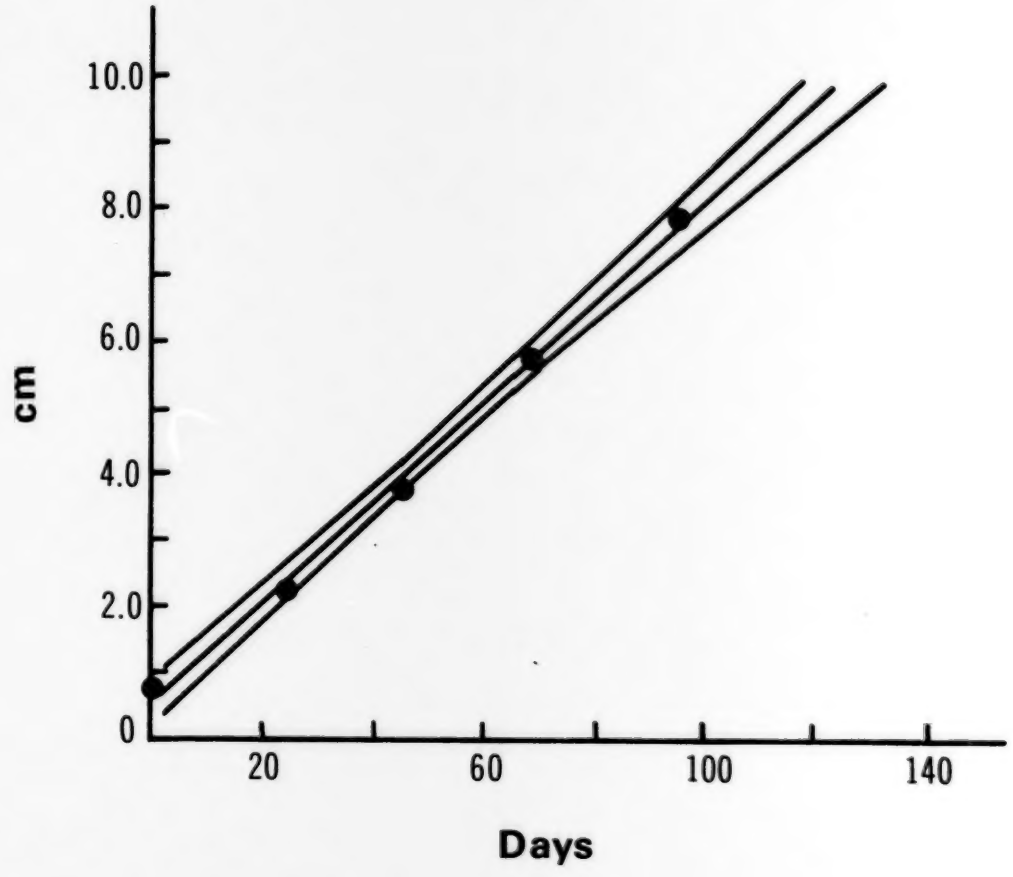

Fig. 29. Mean increase in length of cultured Arenicola cristata during spring and early summer; 95% confidence belts are indicated.

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